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STUDIES IN GALERUCINÆ

The External Morphology of *Galerucella birmanica* (Jacoby), Coleoptera Polyphaga, Phytophaga, Chrysomelidæ, Galerucinæ

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Introduction

Galerucella birmanica is a very serious pest of water-nut or Singhara (Trapa bispinosa) and has been recorded from the following places in India:— *Central Provinces:* Sondad, Bhandara and Shakartala; *Bihar:* Muzaffarpur; *Assam:* Kalsi; and *United Provinces:* Cawnpore. In various ponds round about Nagpur in C.P and Aligarh in U.P. this pest has been found causing considerable damage to the Singhara crop by the author.

Galerucella birmanica is a pest of great economic importance as Singhara forms a staple diet of the inhabitants of many poor villages in various parts of India. The damage is done only to the leaves of the plant and the larva as well as the adult are destructive, the former causing greater damage than the latter. Tender leaves are mostly affected; as a result, the growth is much retarded and the number of fruits per plant is greatly reduced.

The sub-family Galerucinæ to which this pest belongs is one of the largest sub-families of Chrysomelidæ comprising about four thousand species varying in size from 2 mm. to 18 mm. Out of these 417 have so far been recorded within the faunistic limits of India, Burma and Ceylon. The great majority is dull brown but there are many which exhibit brilliant coloration. They are all without exception phytophagous.

In a previous paper (Khatib, M. H. 1934, *Indian Journal Agric. Sci.*, Vol. IV, Part IV) a detailed account of the life-history and biology of this pest together with the external morphology of the larva and pupa was described. The present contribution is a continuation of the work mentioned above. As has been rightly pointed by Ayyar[3] very little work has been done on the life-histories and habits of Galerucine beetles. Our knowledge of the structure of the members of this family is still more scanty and hence no apology is needed in attempting a detailed morphological and anatomical study of one of the members of this very important sub-family. To the best of the author's knowledge no attempt has so far been made in India to study the morphology or the anatomy of any member of this sub-family.

Zorin[78] has published an account of the Biology and Morphology of *Galerucella vibroni* and Servidei[58] has given an account of the Biology and Morphology of *Galerucella nymphaeæ*. The present work has been undertaken to throw some more light on the structure of these important beetles, and consequently the external morphology (and the internal anatomy, which will be shortly published) has been studied in much greater detail than hitherto attempted.

The Galerucinæ has been defined by Maulik[44] thus: "Terrestrial Coleoptera; phytophagous; all tarsi with four segments, at least the penultimate bilobed and the last or claw-segment arising from the base of the previous segment; body oblong, mouth parts without a snout and not ventrally situated; antennæ not modified in a manner to which the term "elbowed" is applied; the roots of the antennæ situated close together in front between the eyes; the posterior femur, when thickened, without a jumping organ".

It may be noted here that in the larva sent to Maulik by the author he finds (44: p. 32) a difference in the arrangement of sclerites of the third stage larva from Aligarh and that from Nagpur. This difference in the general scheme of the arrangement of sclerites of the same stage larvæ from the two localities is due to the fact that the Aligarh specimen was fixed at the end of the stadium when it was about to pupate and the Nagpur specimen was fixed near about the middle of the stadium. Unfortunately Maulik has not

noted the differences in detail except that " in the Aligarh specimen of the third stage larva, the general scheme and arrangement of sclerites seems to have almost disappeared ". Since there is much increase in the size of the third stage larva before pupation, most of the sclerites become obliterated and the setæ also get widely separated one from the other when the larva fixes itself to the surface of a leaf for pupation. Same is true of the first and second stage larvæ prior to ecdysis. Immediately preceding moulting the larvæ get slightly modified.

II. Historical

The sub-family Galerucinæ is one of the two families of the division Trichostomes which is one of the five divisions of the large family Chrysomelidæ of the super-family Phytophaga belonging to the sub-order Polyphaga of the order Coleoptera. Maulik[44] has given reasons (pp. 65–66) for treating Galerucinæ as a sub-family and not as a family as proposed by Boving and Craighead (*Entomologica America*, XI, pp. 1–35, 1931).

The genus Galerucella was first proposed by Crotch (*Proc. Acad. nat. Sci. Philad.*, pp. 19–33, 1873) thus separating it from "Galeruca. In the year 1875 the 11th volume of the Genera was published (Chapuis-Historie Naturelle des Coleopteres, etc.," by Lacordaire) which formed the basis of the table of genera given in the classification of Coleoptera of North America. In 1922 Laboissiere ('Ravue du groupe des Oidietes africans," *Ann. Soc. Ent. Fr.* XC, pp. 193–234), made nymphæ the geno type of a new genus which he called Hydrogaleruca. Maulik[44] thinks that the two genera, Hydrogaleruca and Galerucella, should be regarded as synonyms and fixes *Chrysomela nymphææ*, Linn. as the genotype. Altogether five species of this genus have so far been recorded from the Indian region: *Galerucella placida* described by Baly (*Cistula Entomologica, London*, ii, pp. 369–388, 1878); 2. *Lochmæa birmanica Jac.* (Galerucella birmanica (Jacoby)) described by Jacoby (*Ann. Mus. Stor. nat. Genova*, xxvii, 1889, p. 215). The other three species *viz.*, *Galerucella aureta*, *G. aludela* and *G. digambara*, have been described by Maulik (*Fauna of British India—Galerucinæ*, 1936).

G. birmanica was first described by Jacoby in the year 1889 under the name *Lochmæa birmanica* and he records its presence on the leaves of *Euryale ferox*—a water plant. The original description of Jacoby has subsequently been modified by Maulik in his Fauna volume referred to above.

It was possibly on account of the fact that the species was first recorded on the leaves of *Euryale ferox* that Lefroy, when he found it feeding on the leaves of water-nut regarded it to be a new species and provisionally named

it *Galerucella singhara* (*Mem. Dept. Agric. India, Entom. ser.* Vol. III, 1910) or possibly Jacoby's original description might have escaped his notice. Mohd. Afzal Husain and Syed Abdulla Shah[31] in their work on *Aulacophora abdominalis* (*A. fevicolis Lucas*) have mentioned that the occurrence of *A. abdominalis* on Trapa bispinosa was a case of " mistaken identity, because the real pest of this plant *Galerucella singhara* Lefroy resembles closely *A. abdominalis* in general form and colour ". Maulik[44] is correct in so far as he denies any resemblance of the former with the latter but when he goes further and says that " Lefroy did not publish any description of such a species of Galerucella " he is mistaken. Lefroy[41] has certainly described the life-history of this beetle and it appears to me that Maulik somehow missed this publication during the course of the preparation of his Fauna volume. I find no reference to this work in the bibliography he has given, and further, in the introductory part of the Fauna volume where he has given notes on the earlier stages, life-histories, habits and food plants, he writes a few lines (page 32) about *Galerucella placida* Baly (Syn. *G. rugosa* Jacoby) and has referred to Lefroy's Indian Insect Life, where just a brief reference has been made to this species, and makes no mention of Lefroy's account of the life-history of this pest which has been described in much greater detail together with that of *G. birmanica* (Syn. *G. singhara*) in the publication referred to above. Lefroy has given only the general characters of *Galerucella birmanica*. Unfortunately I could not get his type specimen but from the general form and colour of the body of the adult and the larva, so beautifully illustrated in his coloured plates, and from the parallelism in the life-histories of *G. birmanica* and *G. singhara* I am almost certain that *G. singhara* and *G. birmanica* are one and the same species. Further, the colour plates depicting the life-cycle of *G. singhara* together with the nature of damage done to a singhara leaf, leave no doubt in my mind as to the identity of *G. singhara*.

III. Material and Method

The insects were collected from various tanks round about Nagpur. The material was compared with the identified specimens so very kindly returned back to me by Professor Maulik. The specimens sent to Professor Maulik from Aligarh and those sent from Nagpur were identified by him to be one and the same species.

Both preserved and fresh material was used for external anatomy but it was found that material killed in hot 70% alcohol yielded the best results. Dissection of the chitinous parts was carried out by putting the specimens, after they were thoroughly soaked in water, in 10% caustic potash and gradually boiling them in a water bath. After clearing all the soft parts

the specimens were transferred direct to glacial acetic acid while still hot and allowed to stay there for about an hour and examined. Afterwards they were put in Terpineol. This oil has an advantage over Cederwood oil or Clove oil in that it does not make the chitin brittle and keeps it pliable and easy to handle for further examination and dissection of various parts. All dissections were carried out under a powerful Zeiss dissecting microscope and where it was found necessary with the help of a monla lamp. All drawings, except otherwise mentioned, were made under camera lucida and a few with the help of pan phot. They were further checked with the terpeneol kept material under a dissecting microscope as well as under an ordinary compound microscope. This is of very great importance as in some cases due to overlapping and flattening of some parts consequent upon mounting on a slide the correct relations of various parts to one another cannot be made out and the object cannot be seen in relief. Whereas a dissecting microscope allows for manipulation, and the particular object can be viewed from all angles and its relationship to other parts can be made out with certainty. For the study of the muscles of the tarsus and antennæ and the structure of pretarsus the material was dipigmented by subjecting it to chlorine vapours and then staining it with Mayer's paracarmine. Mayer's paracarmine is a more penetrating stain than borax carmine. It stained the muscles very satisfactorily as well as the delicate chitinous structures which could not ordinarily be detected in potash preparations.

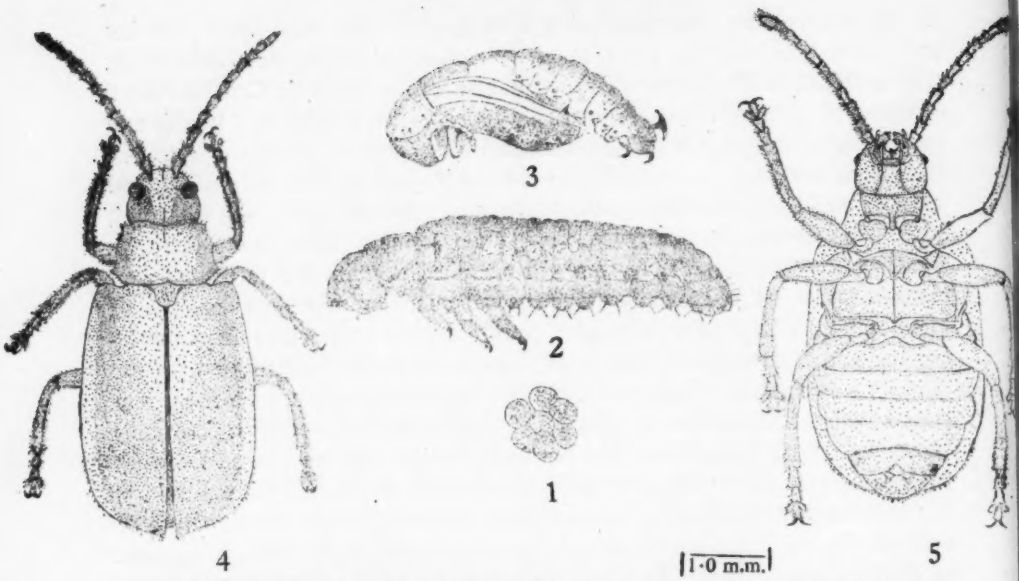
IV. General

The External Morphology of the Imago

Maulik[44] has described the genus Galerucella on pp. 214–216 of his Fauna volume and the species under discussion has been described on pp. 218–219 of the above work.

In one respect where Maulik writes " the punctate surface of the vertex covered with hairs, but not the interantennal areas nor the upper surface of the clypeus " the author differs from him. In this species, as will be found subsequently in the description, the clypeus is divided into an ante- and a post-clypeus. The ante-clypeus is certainly not covered with hairs but the post-clypeus is. These hairs on the post-clypeus are much smaller than those on the vertex and can only be made out under high magnification.

There is no marked difference between the sexes except for the posterior extremity of the abdomen. Comparatively speaking the female is slightly larger than the male and her body is slightly broader. When the beetle is viewed in its natural position (Fig. 4) only the head, the prothorax and the



All the drawings were made under camera lucida and a few with the help of pan phot. FIGS. 1—5 were made by taking measurements.

Magnification ×8.

FIG. 1 Eggs; FIG. 2 Larva; FIG. 3 Pupa; FIG. 4 Imago, dorsal view; FIG. 5 Imago ventral view.

elytra are visible. Between the prothorax and the elytra is to be seen the subtriangular scutellum which is the only part of the meso-cum-metathorax visible from above. The rest of the body is covered by elytra which extend a little behind the posterior limit of the last abdominal segment. The occipital region of the prognathus head is overlaid by the anterior margin of the protergum and a mid-longitudinal line along this portion overlaps a similar line on the posterior region of the head capsule. The filiform antennæ are situated close together in front of the convex eyes and due to the close approximation of their bases constrict that part of the fronto-clypeal area which lies between them. This area shows no difference between the sexes as is common in many Galerucinæ. Out of the five visible abdominal sternites the last shows modifications in the two sexes owing to the exigencies of copulation. The entire body of the beetle is covered with fine hairs which are specially abundant on the ventral surface of the tarsi. This abundant and uniform covering of hairs increases the surface tension and prevents the animal from sinking in water.

V. The Head Capsule

The head (Figs. 6, 7, 8) is rounded, strongly chitinised and is capable of much free movement. The occiput is not a distinctly marked sclerite from the epicranium. The occipital foramen (FM.) is surrounded by a distinct post-occiput (*p.o.*) to which the neck membrane is attached. It is marked off from the occiput by a post-occipital suture (*p.o.s.*) and ends at the posterior tentorial pits (*p.t.*). The post-occipital suture is inflected along its dorsal aspect to form an internal flange upon which are attached the anterior ends of the dorsal prothoracic muscles that move the head. Stickney[70] regards the entire area, extending from the occipital foramen to the vertex, as the occiput. According to this terminology the area labelled as post-occiput (*p.o.*) should be termed as occiput and the post-occipital suture (*p.o.s.*) as the occipital suture. In front of this suture there is a slight indication of the median epicranial suture (*m.e.s.*)—coronal suture of some authors. It is this suture which is seen in line with a faint ridge on the pronotum. As is the case with some Neuropterous and Coleopterous larvæ (Crampton[7]) this suture divides the occiput into two parts.

Dorsal Aspect.—(Fig. 6). In naming the various sclerites of the head I have followed Imms' nomenclature.[33] Hence the interantennal area of Maulik may be termed as frons (*f.*) and his interocular area the vertex (*v.*). It carries the paired antennæ but bears no ocelli. The entire epicranium is closely punctate and thickly covered with hairs except on certain areas, to be referred to presently, and is slightly flattened. The inter-ocular space is slightly depressed, with a median impressed line (*i.o.l.*) which is joined anteriorly by two horizanto-oblique lines (*h.o.l.*). The two antennæ (*a.*) are lodged in antennal sclerites (*a.s.*) provided with a membranous rim all round. The frons (*f.*) is narrowed down due to the proximity of the bases of the antennæ, on each side of which there is an oval elevation (frontal elevations of Maulik—*f.e.l.*), uncovered with hairs. The two areas approximate so closely in the middle line that they form a groove between them, traversed by a deeply impressed line (*f.l.*). The gap between the inter-ocular line (*i.o.l.*) and the frontal line (*f.l.*) is very small and at first glance they appear to be one continuous line but a careful examination of the head capsule, under a powerful dissecting microscope from various angles, reveals their separate identity. The frons is followed by the not very sharply marked off triangular post-clypeus (*p.cl.*) which is also traversed by a median line. This region is again difficult to identify in a mounted specimen, but if the properly cleared head capsule is carefully examined either under a microscope or a dissecting binocular this region at once becomes apparent. Further if this region be viewed from the ventral side through the occipital foramen

a distinct V-shaped line is seen, the arms of the V pointing towards the anterior side of the head capsule where they rest upon the anteclypeus, and the apex of the V points towards the bases of the antennæ. The post-clypeus is thinly covered with very fine hairs, so fine that when under ordinary magnification the hairs of the vertex are clearly visible the post-clypeus looks quite smooth. Its surface is not punctate. The post-clypeus carries on either side a convex process (*p.g.m.*) for articulation with the ginglymus of the mandible. This sclerite is followed by a more or less membranous ante-clypeus (*a.c.l.*) which is smooth and non-punctate. The strongly chitinised labrum is moveably articulated with the ante-clypeus by a distinct clypeo-labral suture (*c.l.s.*). The labrum (*lr.*) does not completely cover the mandibles (*md.*), the latter, when viewed in an untreated head, are seen to be overlapping one another like the blades of a scissors. If the head is turned slightly to the lateral side the anterior tentorial pits (*a.t.*)—the pretentorinæ of Stickney[10]—can be seen lying above and slightly posterior to the condylar articulations of the mandibles. The two lateral surfaces of the head are occupied by the genæ (*g.*). The compound eyes (*e.*) are oval and convex, and are situated dorso-laterally behind the antennæ.

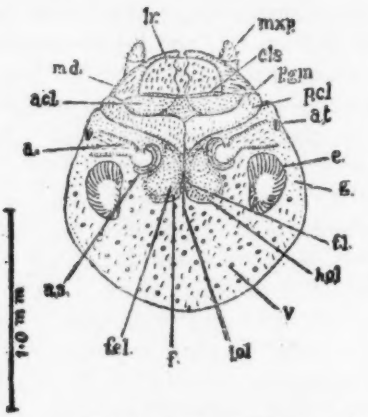


FIG. 6- Head, dorsal view

The labrum (Fig. 7) forms the dorsal wall of the intergnathal preoral cavity. It is more or less a quadrangular structure with a rounded anterior margin. Its dorsal surface is coarsely punctate and covered with hairs. It is moveably articulated with the membranous ante-clypeus by a distinct clypeo-labral suture. The ante-clypeus covers the posterior membranous portion of the labrum. The inner wall of the labrum is membranous and forms the anterior portion of the epipharynx. The labrum-epipharynx is

covered with very long bristles and is provided with a number of sensory patches. Posteriorly the labrum-epipharynx is continuous with the membranous inner surface of the clypeal region of the head. Arising from the

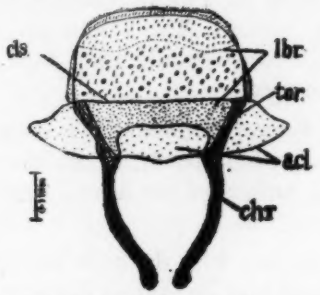


FIG. 7. Labrum, dorsal view

posterior angles of the labrum and attached to the tormæ (*tor.*) are a pair of chitinous rods (*ch.r.*) which project into the ephipharyngeal region and are attached to its lateral margins. The distal ends of these rods approach one another. These rods give support to the pharynx and part of the œsophagus. Revnay[66] regards them to have originated due to chitinization of the lateral walls of the epipharynx. The posterior membranous part of the labrum may be called the post-labrum (Crampton[7]).

Ventral Aspect.—(Fig. 8). The gula (*gu.*) which forms the middle portion of the floor of the head capsule, as in most coleoptera, is clearly

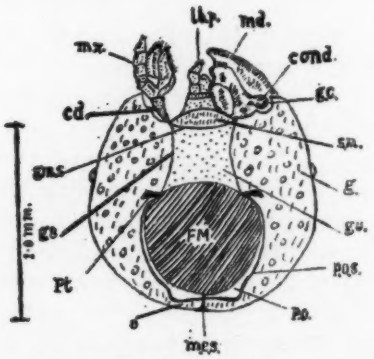


FIG. 8. Head, ventral view

marked off anteriorly from the submentum by the gulo-mental suture (*g.m.s.*) and laterally from the genæ by the gular sutures (*g.s.*). At the base of the gula and in front of the occipital foramen are situated the posterior

tentorial pits (*p.t.*)—metatentorinæ of Stickney. Each gena on either side bears a cavity (*g.c.*) for the reception of the condyle of the mandible. The formation of the gula is associated with the prognathus condition. The head is turned upwards on the neck which results in the true posterior surface of the head becoming ventral. The mouth parts come to be directed forwards and their bases move forwards away from the occipital foramen, and thus the gula is developed.

Hairs, etc., on the Ventral Surface.—The ventro-lateral parts of the genæ are covered with hairs, both the hairs and the punctation are coarser than those on the dorsal surface of the head. There are no hairs on the gula nor its surface is punctate.

At the junction of the gula with the gena is a small socket into which the condyle of the cardo (*cd.*) of the maxilla fits in. In between the attachment of the two maxillæ, the submentum (*sm.*) is articulated by a distinct suture with the gula. The lateral pointed extremities of the submentum fit along the inner margins of the genæ slightly posterior to the articulation of the cardines of the maxillæ.

Endo-Skeleton.—The anterior and the posterior arms of the tentorium do not advance much backwards and forwards respectively from their points of origin and do not meet each other. The dorsal arms of the tentorium are not at all developed. On the whole the tentorium is a rudimentary structure in this beetle.

VI. Appendages of the Head

1. *The Antennæ.*—(Figs. 9, 10). The antennæ are to be regarded as the first of the appendicular organs of the insect head, and are thought to belong to the preoral part of the head (Snodgross[67]). The two antennæ are situated close together at the anterior region of the head. Since they are never situated on the true frons the approximation of their bases greatly narrows down the frontal area. Each antenna is lodged in its socket provided with a distinct antennal sclerite with which it is moveably articulated by an arthrodial membrane. The antennæ are filiform and show no sexual dimorphism. Like most of the coleoptera there are eleven segments. As in Pterygota they are of annulate type (Imms[36]). Pedicel or the first annulus (*ped.*) is not very sharply differentiated from the rest of the flagellum.

As is the case with all pterygote insects the annuli of the antennæ are not provided with any muscles and the flagellum and the pedicel as a whole are moved by a flexor and an extensor muscle. The former (*flex.m.ant.*) is the longer of the two and originates from the base of the scape and is inserted

at the base of the pedicel. The smaller extensor muscle of the antenna (*ext.m.ant.*) similarly originates from the base of the scape and is inserted at the base of the pedicel.

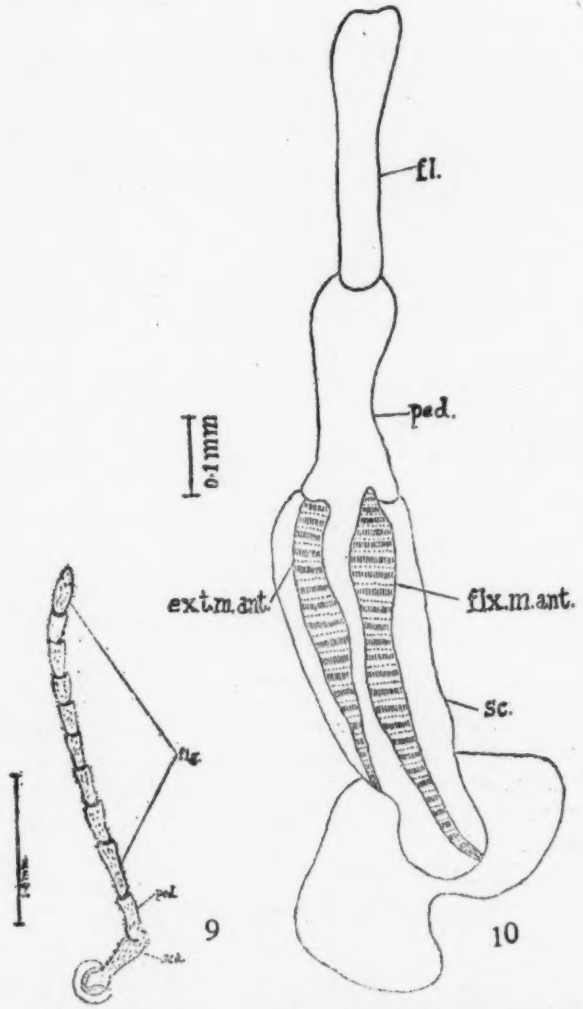


FIG. 9. Antenna FIG. 10. Antenna, stained showing muscle

In an average specimen the total length of the antenna is 3·07 mm. The scape is the longest segment and measures 0·5 mm., next comes the pedicel measuring 0·28 mm. The third segment is the longest of the annuli and

measure 0·42 mm. This is followed by the fourth measuring 0·3 mm., the fifth segment is slightly smaller than the fourth and measures 0·24 mm.; the sixth segment is smaller than the fifth and measures 0·22 mm.; the 7th is equal to the sixth; the eighth segment measures 0·2 mm.; the ninth segment is larger than the eighth measuring 0·23 mm.; the tenth is the smallest segment and measures 0·18 mm.; the eleventh segment is equal to the second segment. All the segments of the antenna are uniformly covered with fine hairs.

2. *The Mandibles.*—(Fig. 11). The mandibles partly form the lateral margins of the preoral cavity and represent the coxopodites of the typical

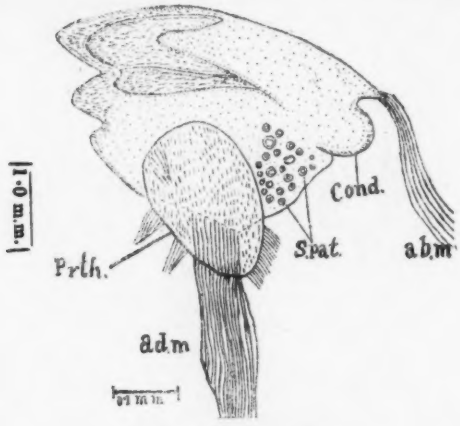


FIG. 11. Mandible.

arthropodan limb. They are strongly chitinized structures capable of great freedom of movement both by the nature of their attachment and the possession of the great adductor and abductor muscles. They are for the most part covered by the labrum. Each mandible is provided with five, more or less blunt teeth, for reducing down the plant food to small bits. The teeth are black in colour. Towards the oral side of each mandible there is attached a moveable prostheca (*prth.*) provided with four tufts of highly specialised setæ and a uniform covering of small hairs. Each mandible is attached by a more dorsally placed ginglymus (*ging.*) to a convex process of the post-clypeus and by its more ventrally placed condyle (*cond.*) with the concavity of the gena. Each jaw is moved by powerful adductor (*ad.m.*) and abductor (*ab.m.*) muscles. The former is much more strongly developed than the latter and consists of several bundles of fibres inserted on a large podeme attached at the inner angle of the mandibular base. The postero-

ventral side of each mandible is much less chitinized and is provided with a number of sensory patches (*s.pat.*). The abductor muscle is attached directly to the mandibular base along its outer angle. When the animal is resting the adductor muscle is kept in a state of contraction and the abductor is kept relaxed, as a result the mandibles always overlap one another.

3. *The Maxillæ.*—(Fig. 12). The maxilla is to be regarded as the highly modified derivative of a walking limb. Each maxilla is articulated by its cardo-condyle (*c.c.*) to the head capsule at a little depression placed slightly outer to the lateral limit of the submentum at the extreme anterior limit of the gular suture. The cardo (*ca.*) shows no division into basi-cardo and disti-cardo.

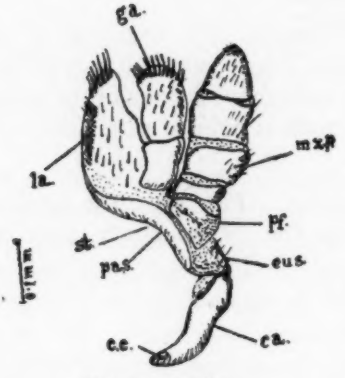


FIG. 12. Maxilla.

The stipes (*st.*) is divided by a longitudinal ridge into eustipes (*eus.*) and parastipes (*pas.*) and forms the main portion of the body of the maxilla (Crampton[9]). The palpifer which bears the four jointed maxillary palp (*max.p.*) is intimately united with the true stipes (eustipes). The lacinia (*la.*) is a blade-shaped structure bearing a tuft of very thick and strong setæ at its tip and a number of smaller setæ along its inner margin. The galea (*ga.*) is two jointed and partly covers the lacinia. It also bears a tuft of strong bristles at its tip.

4. *The labium.*—(Fig. 13). The labium is formed by the fusion of the appendages of the labial segment serially homologous with those of the maxillary segment and forms the floor of the preoral cavity. Primarily the labium is divided into a proximal prementum (*pm.*) and a distal postmentum, the line of division between the two being called the labial suture (Imms[33]). As in many insects a distal sclerite or mentum (*mn.*) is developed immediately behind the labial suture and the proximal area of the original post-

mentum is the submentum (*s.m.*). The submentum is attached along its entire width to the gula and the two outer pointed corners of the submentum are inserted in small sockets placed mesiad to the attachment of the two cardines. As indicated by Crampton[9] in some Coleoptera, the well-developed palpigers (*p.gr.*) approximate mesially and form the greater part of the region called prementum. Due to this mesial approximation of palpigers the ligula becomes crowded and displaced anteriorly and mesially.

The paraglossæ and glossæ are fused into one lobe, the ligula (*lg.*). The lateral portions of the ligula may, however, represent the paraglossæ and the median portion the fused glossæ. The palpiger (*pgr.*) bears a three-jointed labial palp (*lb.p.*).

Hairs, etc.—The submentum is covered by a large number of elongated setæ and this character clearly marks it out from the gula behind. The mentum is the most membranous portion of the labium and is entirely devoid of hairs. The prementum is covered with small hairs and so are the labial palpi.

5. *The Hypopharynx.*—(Fig. 14). The large and membranous hypopharynx arises from the floor of the mouth cavity and is intimately attached

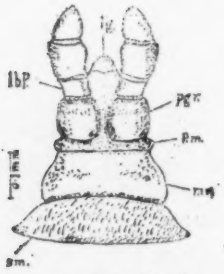


FIG. 13. Labium

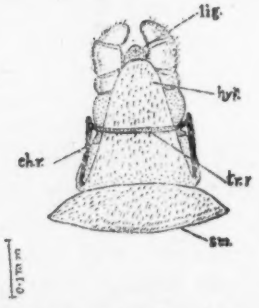


FIG. 14. Hypopharynx attached to dorsal wall of labium

anteriorly to the dorsal wall of the ligula. Posteriorly it lies above the labium, almost covering the latter organ and thus forming the real floor of the preoral mouth cavity. The large size of the hypopharynx may be attributed to the nature of the food plant which is very juicy. The surface is covered by a large number of very fine setæ which are probably gustatory in function. The hypopharynx is supported on either side by a chitinoid rod (*ch.r.*), the hypopharyngeal bar of Dorsey[22], running dorsoventrally across the head capsule and attached on its ventral side to the outer corners of the prementum. These two rods are connected with each other ventrally

by a more or less semicircular transverse chitinous rod (*tr.r.*) which passes below the labium and forms a brace surrounding it ventrally.

VII. The Thorax

1. *General Account.*—The membranous region lying between the prothorax and the head and commonly known as the cervix or neck is without any sclerites. In the natural position of the animal this region is completely covered by the overlapping pronotum. The morphological nature of the neck region of insects is not yet clearly understood but according to Snodgrass[65] there is some embryological evidence to suggest that the post-occipital suture is the line of separation between the labial and the maxillary segments of the head and hence is the only suture of the head having an intersegmental value. Accordingly the post-occiput of the cranium is a sclerotic remnant of the dorsal arch of the labial segment and the region of the neck is supposed to include a posterior membranous part of the labial segment and the anterior part of the prothorax.

The prothorax is the least specialized of the three thoracic segments. It is quite separate from the mesothorax, partly covers the occipital region of the head and is freely moveable. The meso- and metathorax are very intimately welded together and are highly specialized to secure greater mechanical efficiency consequent upon the development of wings. As is the case in Coleoptera the forewings become modified into elytra and take no direct part in flight. As a result the mesothorax is much smaller and less specialized as compared to the metathorax. The endophragmal skeleton of the metathorax is also highly developed in order to provide attachment for the muscles of the hind wings.

2. *The Prothorax.*—(Figs. 15, 16). The pronotum is not divided into separate sclerites and is the most prominent part of the thorax. It is more or less rectangular in shape with a convex dorsal surface. Its anterior border is deflected downwards in order to provide attachment for the shorter muscles connecting the head with the protergum and the latter with the mesotergum. Along the mid-dorsal region of the anterior one third of the pronotum there can be seen a line which is more prominent anteriorly than posteriorly. When the head is in position this line is in exact opposition to the coronal suture of the head. This line is the external manifestation of the mid-dorsal invagination of the pronotum. The lateral margins of the pronotum on either side extend ventrally and are indistinguishably fused with the pleuron of its side. This lateral extension of the pronotum may be called subnotum (*sn.*) and the common sclerite formed by the fusion of the more ventral part of the pronotum with the pleuraon may be termed the

pseudopleuron (*ps.pl.*) (Revnay[56]). The eusternum—basisternum of Snodgrass—is a large sclerite and extends laterally and becomes united with the pseudopleuron. A process (*eus.l.*) extends backwards from the eusternum and is generally called the prosternal lobe. Since it lies between the two coxæ of the fore legs and is of value in classification it has been named inter-coxal process by Maulik[44]. Correctly speaking, it should be called the eusternal lobe since it arises from the eusternum. It is narrower in the male

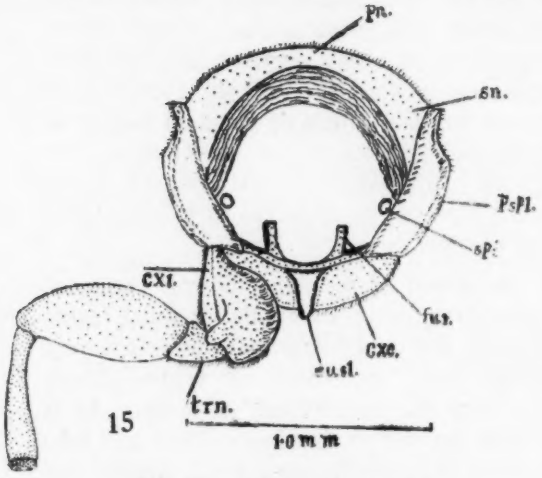


Fig. 15. Prothorav, posterior view

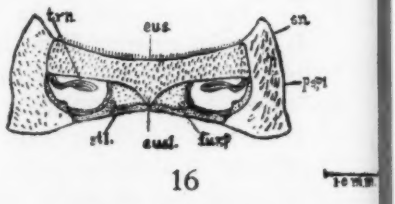


Fig. 16. Prosternum

and broader in the female. Behind the eusternum is that portion of the venter (*stl.*) which bears the furca (*fur.*). Although this sclerite is not clearly marked off from the eusternum but since it bears the furca it represents the sternellum (furcasternum of Snodgrass) of the more generalized insects. The furcal pits (*fur.p.*) are clearly marked out along the inner margins of the coxal cavities. The front coxal cavities are open as the eusternal process (inter-coxal process of Maulik) does not extend so much laterally as to become fused with the pseudopleuraon ventrally. The condition of open or closed coxal cavities has been much used in classification but Maulik[44] does not regard it to be a character of much taxonomic value. The coxal cavities (*c.c.*) are suboval. At the anterior border of each of the coxal cavities lies the trochantin (*trn.*) which is a small and curved sclerite to which are attached the tergal promotor muscles of the foreleg. It receives the condyle of the coxa in its acetabulum. The trochantin has been regarded as an anterior remnant of the coxopleurite by Snodgrass[67]. The inter-coxal process of male is more pointed than that of the female.

3. *The Mesothorax.*—(Figs. 17, 18). This is the smallest of the three thoracic segments and is intimately united with the metathorax. Unlike the wing-bearing segments of most pterygota the tergum of mesothorax is not divided into an anterior plate or notum and a smaller posterior plate or post-notum (pseudonotum of Verhœff or post-scutellum of other authors). Unless as pointed by Snodgross the two small plates joining the mesonotum with the metanotum are rudiments of it. Since it is never present in the

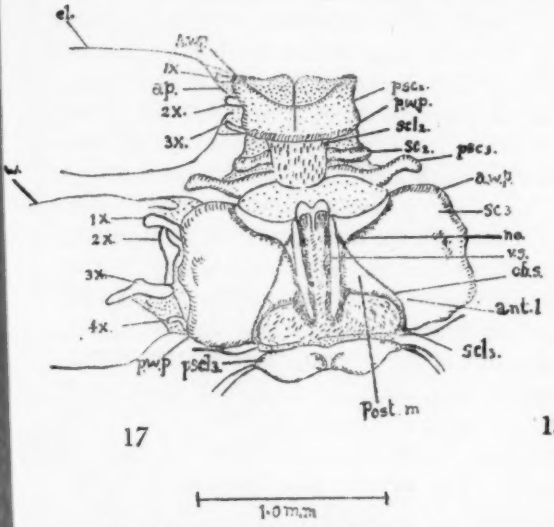


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Fig. 17. Meso- and Meta-tergum

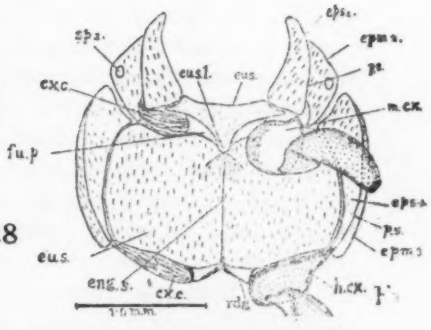

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Fig. 18. Meso- and Meta-sternum

prothorax nor in the pupæ of coleoptera and neuroptera nor in the adult orthoptera and is developed in the intersegmental membrane Snodgrass[60] does not regard it to be a primitive tergal plate. The notum as in most pterygota is divided into prescutum (*psc.* 2), scutum (*sc.* 2) and scutellum (*scl.* 2). With the exception of the last division the entire mesonotum is hidden by the elytra and the pronotum. The posterolateral margins of scutellum overlap the anterior mesal areas of the elytra during rest. There is no postscutellum (postscutum of Snodgrass). The prescutum is a heavily chitinized sclerite and bears a pair of pointed anterior notal wing processes and a pair of blunt posterior notal wing processes. The scutum is widely separated from the prescutum and is overlaid by the scutellum to which it is intimately welded. Imms[33] (p. 481) in his account of the thorax of coleoptera writes "The tergum of both segments (meso- and metathorax)* is divisible

* Words in parenthesis Author's.

into prescutum, scutum and scutellum. The latter sclerite is median in position and divides the scutum into two separated plates". In the species under discussion I find that although the mesoscutellum is median and overlies the scutum yet it does not divide the latter into two "separated plates". Superficially it looks as if the two halves of the mesoscutum lying on either side of the mesoscutellum are "separated plates" but a closer examination of properly cleared material even from the dorsal side shows that the scutum is a continuous plate. If the two sclerites are examined from the ventral side there can be no shadow of a doubt about the matter. This apparent separate appearance of the two plates (although the author had no opportunity of examining allied forms) is probably due to the fact that the scutellum is very heavily chitinized and unless properly decolourized will not show the part of the mesonotum lying below it. Gupta (unpublished) who has studied the morphology of Stromatium barbatum Fabr. confirms my observations. Posteriorly the prescutum sends backwards and downwards two curved processes which intimately unite with the metaprescutum thus uniting the meso- and the metatergum. The two pleural sclerites, episternum and epimeron (*eps.*, *epm.*) are well developed and the pleural suture (*p.s.*) is well marked. It extends dorsally to support the base of the elytron. The pleural ridge of which the pleural suture is the external manifestation is well developed. Ventrally the episternum rests upon the eusternum (*eust.*) and bounds the coxal cavity anteriorly. The epimeron bounds the coxal cavity (*cx.c.*) laterally; posteriorly it is connected with the meta-eusternum mesially and with meta-episternum laterally. The middle coxal cavities are also suboval and contain a small trochantin.

The meso-eusternum is a roughly triangular sclerite with a well developed intercoxal process (*eus.l.*) which is comparatively narrower in male than in female. Antero-laterally the eusternum is suturally united with the mes-episternum. The furcasternum is not clearly marked out as a distinct sclerite but behind the eusternum there can be seen a membranous area into which the furcal pits (*fur.p.*) are lodged. These are the external manifestations of a pair of apophyses which diverge at a wide angle one from the other.

4. *The Metathorax.*—(Figs. 17, 18). This is the most highly developed of the three thoracic segments in view of the fact that the hind pair of wings which are active agents in the flight of beetles arise from this segment. Both externally by the presence of sutures and internally by their ridges this segment shows many peculiarities of its own. The notum is divided into prescutum (*ps.c*3.), scutum (*sc*3.) and scutellum (*scl.*3.). The postnotum (postscutellum, *p.scl.*3.) is well developed.

The prescutum is a transversely elongated sclerite with a large median and convex area and two lateral concave prolongations which hang downwards and again rise up. It is at the middle of these concave prolongations that the scutellum articulates by a pair of forwardly directed processes. These two lateral prolongations of prescutum again rise up in a somewhat convex form and extend backwards to articulate with the scutum of their segment. Along the middle region the prescutum is separated from the scutum by a wide membrane. The scutum is the most characteristic sclerite in Coleoptera and is the largest of all the metathoracic tergites. Its antero- and postero-lateral borders are respectively known as the anterior and the posterior wing processes to which the wings are attached by membranes. Further the scutum is divided into two halves by the anterior prolongation of the scutullum. Its lateral borders are irregular and give attachment to the axillary sclerites. The following sutures and areas can be recognized in the scutum-cum-scutellum part of the notum. In naming these the terminology of Snodgrass[60, 61, 67] has been followed.

(*a*) The scuto-scutellar or V-shaped suture (*v.s.*) lies in the posterior part of this region with the apex directed forwards and divides this region into two lateral scutal plates and the median scutellum.

(*b*) A pair of convergent sutures or notaulices (*no.*) can be recognized arising from the antero-lateral margins of the scutum and extend posteriorly to the anterior region of the scutellum converging along the median line as they do so.

(*c*) A pair of oblique sutures (*ob.s.*) extend from the antero-median region of the scutum and end at the postero-lateral area, thus dividing each scutal plate into an antero-lateral portion (*ant.l.*) and a postero-median portion (*post.m.*).

The metascutellum is followed by the post-scutellum (post-notum *pc.l.* 3) which is a very lightly chitinized sclerite. This sclerite is to be regarded as an expanded acrotergite of the first abdominal segment extending forward to the margin of the metatergum as the postnotal plate (Snodgrass,[60, 61, 67]). This sclerite is intimately attached to the first abdominal segment behind and the scutellum in front and consequently the first abdominal segment comes in very close association with the pterothorax. The post-scutellum carries a very well developed phragma.

The episternum (*ep.s*3.) and epimeron (*epm.*) are quite distinct ventrally but as they extend dorsally the epimeron which is a much smaller sclerite reduces down to a mere line along the outer border of the pleural suture. Dorsally the pleural ridge gives support to the base of the wing and ventrally

it gives out a process to which the outer end of coxa is fixed. The pleural suture and the pleural ridge are well developed.

5. *The Sternum.*—There is no presternum and the eusternum (basisternum) is a very large sclerite, the furca arises from a ridge (*rdg.*) on its posterior part and the sternellum (furca-sternum) is not recognizable as a separate sclerite. The metafurca is highly developed (Fig. 19). It arises as a single long stem and is strengthened by a ventral median rib. There are no free laminæ. The narrowness of the base of the furca could be attributed to the proximity of the meta-coxal cavities. Recently the meta-furca of Coleoptera has been made the subject of a comparative study by

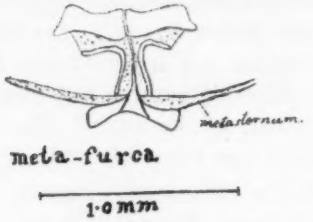

Fig. 19. Meta-furca

Crowson[20] and he finds that the furcæ of Blattidæ, Isoptera, Mecoptera, Trichoptera, Lepidoptera, Psocoptera, described in recent literature do not approach the Protocoleopterous furca and hence the beetles remain as an isolated group. He thinks that " if one could understand why the ancestors of Coleoptera developed such an unusual type of furca, the essential nature of the largest order of insects would be better understood ". The meta-eusternum is very intimately attached by a tough membrane with the meso-eusternum. A round blunt tongue-like process given off from the meta-eusternum meets the intercoxal process of the meso-eusternum. This process again is broader in the female than in the male as is the inter-coxal process of the preceding segment. The sternal elements of the meso- and the metathoracic segments are so intimately united as to form a sort of plastron. An enigmatic longitudinal suture (*eng.s.*) represented internally by a sharp ridge extends forward from the middle of the hind margin of the meta-episternum and stops short near the tongue-like process of this sclerite. The ridge of this suture is continuous with the ventral ridge of the furca. The hind coxal cavities are elongated and so are the coxæ. The coxæ partly overlie the first (morphologically the third) abdominal sternite in its depression and are fixed, as in most Coleoptera, at their outer ends to a process arising from the end of the pleural ridge, already referred to, and at the inner end to a sternal process. Thus they rotate about an axis through these two fixed points.

6. *The Legs.*—(Figs. 20, 21). As in the phytophaga the tarsi are apparently four jointed. The three pairs of legs are more or less equal in size and the hind femora show no enlargement. The coxæ (*cx.*) of the middle and the forelegs are oval and have a greater freedom of movement than those of the hind legs which are transversely oval and restricted in their movement

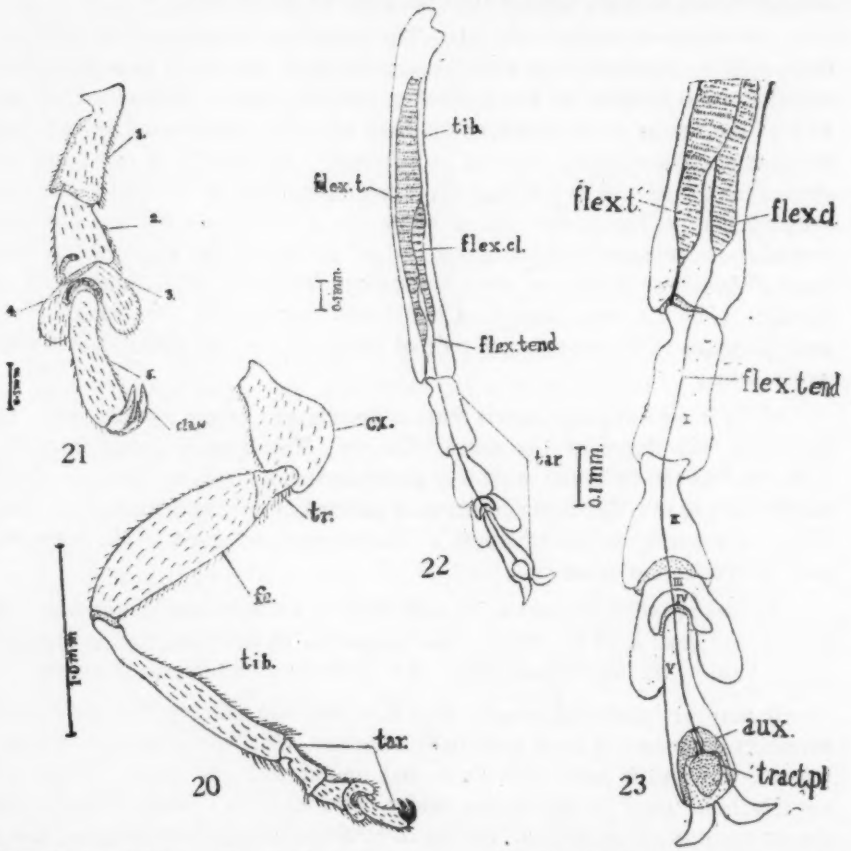

FIG. 20. Leg entire, FIG. 21. Tarsal segments. FIG. 22. Leg showing tarsal muscles and structure of pretarsus. FIG. 23. Part of above, highly magnified.

due to the nature of their attachment. The coxa is followed by the triangular trochanter (*tr.*) which gives attachment to the femur (*fe.*) which is the stoutest part of the leg. The femur is followed by the cylindrical tibia (*tib.*). The hairs on the distal region of tibia resolve themselves into fairly stout and elongated bundles along the two corners. The tibia is followed

by a tarsus (*tar.*) whose third segment is bilobed and receives the small fourth joint, which in turn articulates with the claw-bearing fifth joint. All the tarsal segments are covered by cushions of fine pubescent hairs on their ventral side. The claws are curved and appendiculate. Each claw is provided with a fine claw-like projection coming out from the inner side of its base which is much smaller than the claw which bears it.

The Pretarsus.—(Figs. 22, 23). The pretarsus (transtarsus of Hansen) composed of the claws and their associated basal structures is supposed to represent the dactylus of the typical crustacean limb. It arises from the end of the tarsus by a membranous base which is invaginated in the last tarsomere. There is no trace of an arolium. The cavity of the pretarsus is continued into the two lateral claws which are hollow structures and are appendiculate. The claws rest dorsally on a small median process (the unguifer of Snodgrass) of the distal end of the last tarsal segment. At the bases of the claws is situated the tractor plate (*tract.pl.*)—the unguitractor of Snodgrass—of an oval shape. Closely associated with the tractor plate and the bases of the claws are a pair of lateral plates (the auxilliæ of Snodgrass, *aux.*).

As in insects and myriapods there is associated a single muscle with the pretarsus, the flexor of the claws (*flex.cl.*). This muscle arises from the distal end of the tibia and is closely associated with the flexor muscle of the tarsus (*flex.ta.*). The flexor of claws is inserted on a long tendon (*flex.tend.*) which traversing the entire length of the tarsus is attached to the proximal end of the tractor plate.

Associated with the tarsus as well there is a single muscle, the flexor of the tarsus (*flex.t.*). This muscle also originates in the tibia and is inserted at the base of the first tarsal joint. An extensor of the tarsus is absent.

It may be incidentally mentioned here that the character of the Phytophaga as described in most systematic accounts, *viz.*, " tarsi apparently four-jointed, the fourth joint very small and united with the fifth " does not entirely hold good in the species under consideration. While investigating the structure of the pretarsus, for which bleaching and staining was necessary it was found that the fourth joint was not fused with the fifth as commonly believed to be the case but is seperated from it by a very distinct membrane. The fourth joint on the other hand is very intimately welded to the third tarsal joint.

7. ***The Wings and their Articulation.***—The first pair of wings is modified into elytra which play no active part in flight and serve to protect the greatly folded and delicate second pair of wings during repose. The anterior margin

of the elytron (Fig. 17) tapers abruptly downward into an apophysis (*ap.*) which is connected to the first axillary plate (1X.) and to the anterior wing process (*a.w.p.*) of meso-prescutum by a membrane. The second axillary plate (2*x.*) embedded in the alar membrane connects the apophysis with the wing process of the meso-episternum. This is the continuation of the pleural ridge and suture and is known as the pleural wing process. The third axillary (3*x.*) connects the wing to the posterior notal wing process (*p.w.p.*). The outer margin of each elytron during repose covers the pleural region. The elytra are highly chitinized structures with a number of coarse punctures. They are uniformly covered with hairs and have lost all traces of veins.

The second pair of wings is borne on the metathorax and are an active agent in flight. In repose the wings are folded in a complex manner and are completely covered by the protecting elytra. The axillaries which participate in the formation of the wing joint are much larger than those of the elytra. Each wing is attached to the anterior and the posterior wing processes of its side by a broad alary membrane and is clothed with very fine hairs. The alary membrane is strengthened by four axillaries (pteralia) which conform to the typical number of axillaries present in insects. Below, the wing articulates with the pleural wing process. The first axillary (1X.) forms the anterior hinge plate and articulates with the anterior wing process of the scutum and is associated with the base of the subcostal vein though not actually united with it. The second axillary (2X.) is somewaht obliquely hinged to the lower mesial border of the first axillary anteriorly, and with the third axillary posteriorly. It is related to the radial base. The third axillary (3X.) is a horizontal sclerite and lies in the posterior part of the articular region of the wing. On this sclerite is inserted the flexor muscle of the wing. Articulating with the posterior wing process of the scutum is a very small curved fourth axillary (4X.) which supports the anal region of the wing. There is a wide membrane intervening between this axillary and the third. The fourth axillary has been regarded as a detached piece of the third axillary by Snodgrass[67].

8. *The Wing Venation.*—The wing venation is of the Cantherid type. As a result of folding the veins in the apical area of the wing tend to disappear and the disappearance of some of the proximal ends of the principal veins as well can also be explained on this account. In naming the various veins the author has followed Forbes[23]. The Costa, Sub-costa, Radius and Cubitus are crowded at the costal border of the wing. The main stem of the media has been dropped out. The anal veins are also crowded anteriorly thus leaving the lower portion of this area without any veins with only the

fourth anal lying at the proximal most border. This vein-free area together with the fourth anal is completely folded during rest. *R*1. is enormously thickened so as to obscure the Sub-costa (*Sc.*) which fuses with it distally whereas proximally the Sub-costa fuses with the Costa.

The Principal Veins and their Branches.—(Fig. 24). THE COSTA (*c.*). The costal vein lies along the costal border of the wing and ends at about the middle of the anterior margin.

FIG. 24. Wing showing venation

THE SUB-COSTA (*Sc.*). The second vein is Sub-costa and is concave, lying in a depression on the surface of the wing. Proximally the base of the Costa and the Sub-costa are indistinguishable and distally the latter merges with the Radius.

(iii) THE RADIUS (*R.*). The third vein is the radius and is strongly convex. According to Forbes it is the *R*1. which is continued in the common cavity. The basal portion of the Radial Sector (*Rs.*) disappears leaving a small portion only as a backwardly directed spur, the radial recurrent (*Rr.*). The Radial cross vein (*r.*) extending from *R*1. to *Rs.* is present.

(iv) THE MEDIA. The main stem of media has been dropped out. The two main branches that remain have been regarded M1 and M4 by Forbes[23] and M1 + 2 and M3+4 by Wilson[77]. In the species under discussion the branch M1 (Forbes) or M1+2 (Wilson) is seen to bifurcate at the distal region of the wing, although the two branches are very faintly outlined. According to Lameere's diagram of the hypothetical scheme of wing venation figured by Imms[32, 34] the main stem of the media divides into an anterior convex MA and a posterior concave MP branch. Each of these two branches again sub-divide into two each. In *G. birmanica* two distinct branches of Media, isolated one from the other owing to the atrophy of the main stem, are seen. The anterior branch is very faintly developed and divides into M1 and M2 and may be regarded MA. The posterior branch may be regarded MP. The latter bends sharply downwards and coalesces with the Cubitus, the combined branch thus formed may be regarded Cu + MP (M4 + Cu of Forbes). It reaches the lower margin of the wing. Of the two branches

of MA, MA1 reaches the apical margin of the wing whereas MA2 stops in the middle of the apical area. But as has been pointed out by Imms[32] the constitution of media needs further investigation. A very faint radio-medial cross-vein (*r.m.*) lies between RS and MA. There is also present a very faint medial cross-vein (*m.*) between MA and MP. A distinct arculus (*arc.*) between the Cubitus and Radius is present.

(v) THE CUBITUS. According to Tillyard and other authorities the cubitus is primarily a three-branched vein and the branch Cu2 arises much nearer the base; further forward the branch Cu1 divides into Cula and Culb. In the wing under consideration the Cubitus (*Cu.*) appears to be a single vein unless the first anal (1A.) is regarded to be a branch of Cu., which on the examination of the fully developed wing appears to be highly improbable; because 1A to be regarded as a branch of the Cubitus should arise very close to its base whereas it is quite separate from the Cu. and is only connected to it by a very faint cross-vein. Even this cross-vein is not present in all the wings examined. In some it is not at all developed and 1A ends quite blindly at its posterior end. The Cubitus coalesces with the media and the combined vein reaches the margin of the wing where it tapers to a fine line.

(vi) THE ANALS. There are present three very distinct anal veins in the lower margin of the wing and a broad but lightly chitinized fourth anal along the posterior margin. The first anal (1A) has lost its proximal portion and is connected to the cubitus by a cubito-anal cross-vein (*cu.a.*). It is also connected by a cross vein (*cv.*) with the combined branch of 2A and 3A. Distally it reaches the wing margin. The second (2A) and the third (3A) anals are separate promxially but in the middle of the anal area the two coalesce to form a single vein which does not quite reach the wing margin.

9. *The wing-folding Pattern.*—In studying the wing-folding pattern and naming the various areas Forbes'[24, 25] system has been followed. Fig. 25 shows the wing of the right side when held in repose. The wing was taken undisturbed and sketched under camera lucida. The position of the visible folds in relation to the wing margins and visible veins was laid down. After this the outer part of the wing was unfolded and the folds as they became gradually visible were plotted out. In Fig. 25 the margins are represented by thick lines, the veins by double lines with cross bars and the visible folds by dotted lines. In Fig. 26, the areas of the wing which are reversed in folding are represented by fine stippling. The following reversed areas are recognizable: Axillary (J); Anti-median (C); Pivot (D); Principal (H); First Costo-apical (E); Median (A); and First Anal (G). In between these folded

areas the following upright areas can be recognized: First dorso-apical (*x*); Stigmatal (R); Central (S); Oblong (W) and First Costal (P).

FIG. 25. Wing folded

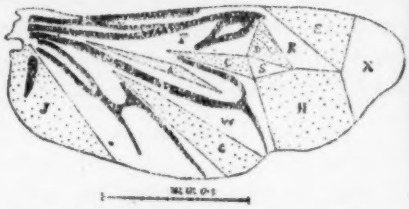

FIG. 26. Wing showing folding pattern

10. *The Spiracles.*—(Fig. 27). The spiracles are generally regarded to be primitively situated in the pleural region of the segment to which they definitely belong and from this position they are assumed to lie either forward or backward in relation to their segment. But the question of the primitive situation of spiracles and their subsequent secondary positions cannot yet be regarded as settled. Most of the insects whether larvæ or adults

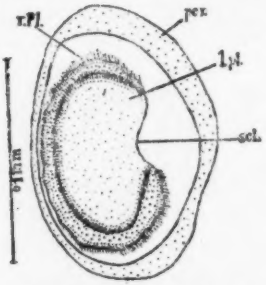

FIG. 27. Thoracic spiracle

are never provided with more than two pairs of thoracic spiracles and it was generally regarded that these spiracles belonged to the mesothorax and the metathorax. In many cases where the spiracles are situated on the prothorax, they were believed to have migrated forward from the mesothoracic segment. Recently Keilin[39] in his valuable contribution has brought forward evidence to show that primarily the spiracles are intersegmental in position at least so far as the larvæ of Diptera, Aphaniptera and the great majority of holometabolous insects were concerned. The two thoracic spiracles present in most insects are regarded by him to be prothoracic and metathoracic and the mesothorax according to him is " not only devoid of spiracles (functional and non-functional) but also of all tracheæ which should belong to it; in other words, it is completely devoid of its tracheospiracular metamere, and the thracheæ which are found within this segment

are derived from the prothoracic and metathoracic spiracles". The presence of eleven body segments and nerve ganglia (when the ventral nerve cord is not condensed) and the maximum number of ten spiracles (functional and non-functional) and the tracheo-spiracular mechanism in dipterous larvæ, according to Keilin, suggest that the primary position of spiracles is inter-segmental " as eleven segments provide only ten intersegmental spaces ". Consequently one segment is left without spiracles. He envisages the migration of spiracles in two opposite directions, the first pair moving anteriorly and the other nine pairs posteriorly. The result is the presence of spiracles on the prothorax and the metathorax, the mesothorax is thus deprived of spiracles. If Keilin's views be accepted the two spiracles in the thoracic region of *G. birmanica* belong to the prothorax and the metathorax.

The first pair of spiracles in *G. birmanica* is situated ventro-laterally in the membrane connecting the pro- and the mesothorax and is not visible from the dorsal side. The second pair of thoracic spiracles is situated in the membrane below the mes-epimeron. The latter are the largest of all the spiracles. Both the thoracic spiracles are similar in structure and the closing mechanism. Each thoracic spiracle (Fig. 27) is situated on a peritreme (*per.*) and as is the case with the thoracic spiracles of most insects, is provided with an external closing apparatus in the form of a pair of moveable right and left lips (*r.lp., l.lp.*) united ventrally in a sclerotic lobe (*Scl.l.*). Both the lips are fringed with very fine hairs. By the approximation of the lips the aperture between them is closed. The presence of fine hairs fringing the lips of the spiracles serve to keep any foreign particles entering the tracheæ when the spiracles are open, thus preventing the system from clogging.

VIII. The Abdomen

The first abdominal segment is very intimately attached to the metathorax and its tergite overlaps the meta-postscutellum to the anterior border of which it is attached by a tough membrane. The second abdominal segment, like the first, is also very small and is attached to the first by a tough inter-tergal membrane. After the fourth the successive abdominal segments show a progressive narrowing in their width as a result of which the abdomen has a tapering appearance, each abdominal segment, from the first to the seventh, is provided with a pair of spiracles. The first abdominal spiracle is the largest of the series. It is also larger than the first thoracic spiracle but smaller than the second thoracic one. It is situated in the pleural region anterior to the line drawn across the middle of the first abdominal segment. Second, third, fourth and the fifth are nearly equal and are situated in their respective pleural regions. The sixth spiracle is smaller than the preceding

four and the seventh is the smallest of the series. The second and the third spiracles are situated in the anterior half of their segments. Fourth to sixth in the middle of the segment, but the seventh is different from the others in that it is situated more dorsally on a raised papilla in the anterior half of its segment. All the abdominal spiracles are alike in their shape and structure and are provided with an internal closing apparatus (Fig. 28). This consists of a pair of chitinized plates (*ch.pl.*) guarding the trachial orifice (*tr.o.*) which is situated at the base of an open atrium (*atr.*).

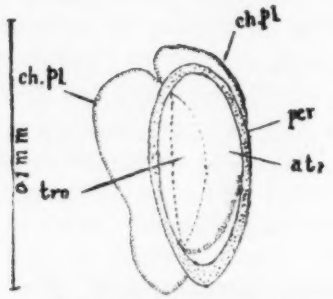

FIG. 28. Abdominal spiracle.

1. *The tergites.*—In both the sexes seven abdominal tergites are visible from the dorsal side. The last tergum is subtriangular. The third tergum is the largest and the succeeding tergites are subequal. All the seven tergites are separated one from the other by inter-tergal membranes and show no telescoping in either sex.

2. *The Sternites.*—Seen from the ventral side, five abdominal sternites are visible. The sternites of the first two abdominal segments have disappeared due to the great enlargement of the metasternum. The first abdominal sternum (actually the third) has a depressed anterior margin on which the hind coxæ rest and is very firmly attached to the metasternum. A conical evagination from the first abdominal sternum fits very closely into an emargination of the metasternum. This evagination is narrower in the male than in the female.

3. *The terminal abdominal segments.*—(*a*) The Female (Figs. 29, 30, 31). The seventh, eighth and ninth segments have been greatly modified. The membranous area surrounding the anal aperture has been regarded as the 10th segment (Snodgrass[66, 67]). The seventh tergum (7 T.) is bent downwards and is conical. It forms a short pygedium. The eighth tergum (8 T.) is completely hidden below the seventh. Although in all the species studied by Tanner[71] he found a spiracle on this segment, there is no spiracle borne

on the eighth segment in *G. birmanica.* Widely separated from the eighth tergum by a membrane is the elliptical ninth tergum (9 T.) whose lateral

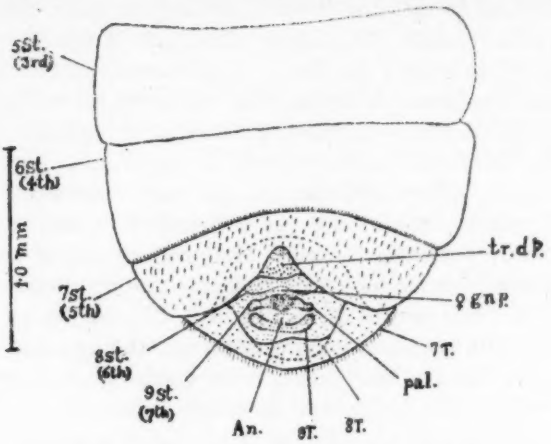

FIG. 29. Posterior extremity of female abdomen

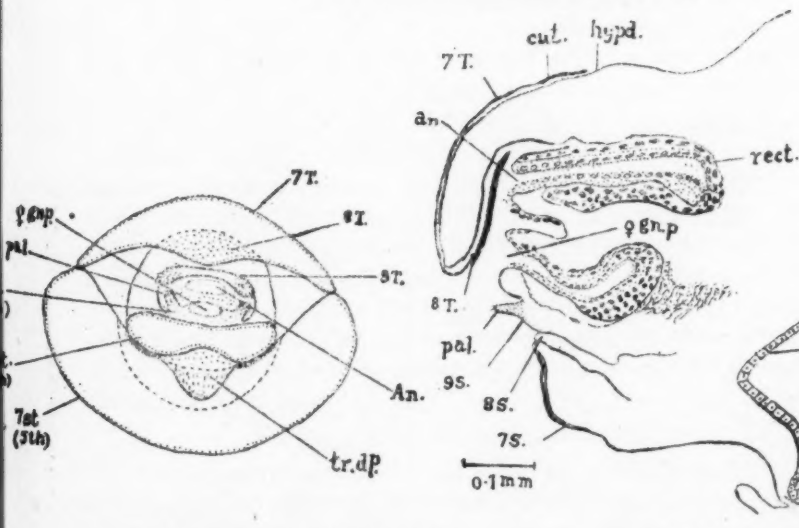

FIG. 30. Diagrammatic sketch of above

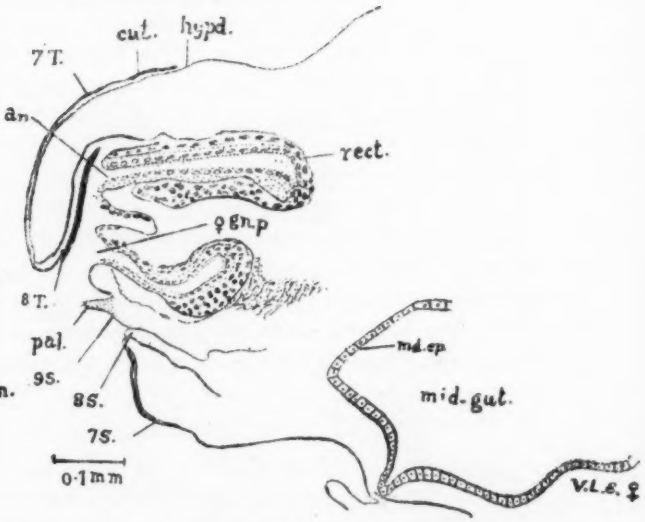

FIG. 31. V.L.S. female abdomen

borders are well chitinized and provided with a number of bristles. Below this segment is the opening of the rectum (*An.*) surrounded by a membrane. The two chitinized lateral plates slightly bend downwards. The fifth

sternum (definitive seventh) has its posterior membranous margin bent inward and backward. It also shows a deep triangular depression provided with very fine setæ (*tr.dp.*) and is so situated as to guide the ædeagus into the female genital pocket. The eighth sternum is in the form of a partially divided plate whose middle portion is membranous, its two lateral portions bear a number of bristles. The gonopore is situated between a pair of widely separated unjointed hollow appendages (*pal.*), the genital appendages or genital palps, borne on chitinous plates. It is now generally accepted (Metcalfe, Pruthi, Imms and others) that they represent the drawn out coxites of the ninth segment and are comparable to the lateral ovipositor lobes of the more generalized insects. On their tips these appendages bear a number of short bristles which are probably sensory in function and serve to feel the surface at the time of oviposition. During the act of oviposition the eighth and ninth segments are protruded and the eggs laid on the surface of the Singhara leaf together with a little quantity of cementing material which glues them to the leaf as well as to one another.

(*b*) *The male* (Figs. 32, 33).—In the male as in the female the eighth tergum is completely covered by the inturned seventh tergum. The ninth

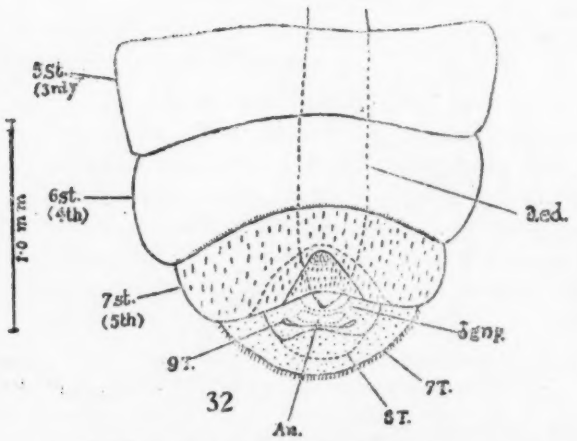

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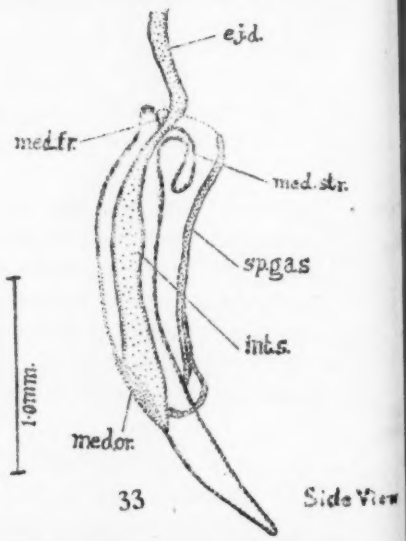
FIG. 32. Posterior extremity of male abdomen



33 Side View

FIG. 33. Aedeagus with ejaculatory duct and associated structures

tergum is represented by a pair of exceedingly small chitinized plates connected with each other by a membrane below which is situated the anus (*An.*).

The seventh sternum (apparently the fifth) has got a ventral membranous fold directed inward and anteriorly like the similar fold in the female. This segment also shows, like that of the female, a deep triangular depression (*tr.dp.*) covered with very fine hairs. Along this area the ædeagus slides during copulation. No parameres or stylets or claspers are developed in connection with the male genitalia. The eighth sternum is not seen. There is an extensive membranous area intervening between the eighth abdominal sternum and the anus. In repose this area is invaginated in the body cavity forming the genital pocket in which the ædeagus (median lobe) lies and is continuous at its base with the former. As suggested by Metcalfe[45] the term ædeagus will be used in the present account since only a single median appendage is present. Sharp and Muir[59] gave the name ædeagus to the median lobe and tegmen together. This is to be discarded. The ædeagus is a well-chitinized, highly developed, long and curved structure (Fig. 33). Contrary to the account of the structure of the male genital tube in Chrysomelidæ by Sharp and Muir[59] there is not the slightest development of tegmen in this beetle. From the basal part of the ædeagus there project downwards a pair of hook-like processes, the median struts (*med.str.*). At the centre of the base of the ædeagus there is situated the median foramen (*med.fr.*) through which the ejaculatory duct (*ej.d.*) enters the ædeagus. The dorsal wall of the ædeagus, in the anterior half of the lower portion, is markedly membranous. It is in this membranous part that the median orifice (*med.or.*) is situated and through which the internal sac is evaginated during copulation. Underlying this membranous area of the median lobe there is a small chitinous sclerite in close association with the internal sac. The long straight ejaculatory duct enters the ædeagus at the median foramen and inside its tubular cavity expands into the internal sac (*int.s.*). The spiculum gastrale (*sp.gas.*) is a lightly chitinized Y-shaped rod, the arms of the Y embrace the genital pocket posteriorly and the stem lies along the ventral side of the genital pocket pointing anteriorly. The spiculum serves for the attachment of the powerful muscles which are used in protracting and retracting the genital pocket and the ædeagus.

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X. Summary

1. The present account is a continuation of work undertaken a few years back by the author.

2. For the first time a detailed account of the external morphology of an Indian Galerucid beetle has been attempted.

3. Evidence has been brought forward to show that Lefroy did describe this species under the provisional name Galerucella singhara and also gave an account of its life-history. Hence Maulik's observation that Lefroy never described any such species is not correct.

4. Only five species of the genus Galerucella have so far been described from the Indian region.

5. It has been found that the mesoscutum which is generally regarded as being split into two separate plates in Coleoptera is one continuous plate in this species and is covered along its middle by the meso-scutellum.

6. The wings are much folded. Their folding pattern and venation has been described in detail.

7. In the male only seven abdominal sternites could be seen and the ædeagus is neither provided with lateral lobes nor there is the development of tegmen.

8. In the female nine tergites and nine sternites can be seen.

9. There is no marked sexual dimorphism.

XI. Lettering

A. .. median.
a. .. antenna.
1A–4 A. .. first to fourth anal.
ab.m. .. abductor muscle.
ad.m. .. adductor muscle.
An. .. anus.
ant.l. .. antero-lateral portion of scutum.
ap. .. apophysis.
a.s. .. antennal sclerite.
atr. .. atrium.
aux. .. auxiliæ.
a.w.p. .. anterior wing process.
C. .. antemedian.
c.c. .. cardo condyle.
cd. .. cardo.
ch.pl. ... chitinous plates.
ch.r. ... chitinous rod.
cl.s. ... clypeo-labral suture.
cond. ... condyle.
cu. ... cubitus.
cu.a. ... cubito-anal cross vein.
cut. ... cuticle.
c.v. ... cross vein between 1st anal and combined branch of 2A and 3A.
cx.c. ... coxal cavity.
D. ... pivot.
E. ... first costo-apical.
eng.s. ... enigmatic longitudinal suture.
epm. ... epimeron.
eps. ... episternum.
eus. ... eustipes.
eusl. ... intercostal process.
eust. ... eusternum.
ext.m.ant. extensor muscle of antenna.
f. ... frons.
fe. ... femur.
f.el. ... frontal elevations.
f.l. ... deeply impressed line of frons.
flex.cl. ... flexor of claws.
flex.m.ant. flexor muscle of antenna.
flg. ... flagellum.
flex.t. ... flexor of tarsus.
flex.tend. flexor tendon.
F.M. ... occipital foramen.
fur.p. ... furcal pits.
g. ... gena.
G. ... first anal. area of wing.
ga. ... galea.
g.c. ... genal cavity.
ging. ... ginglymus.
gn.p. ... genital pore.
g.s. ... gular suture.
gu. ... gula.
H. ... principal.
h.o.l. ... horizanto-oblique line.
hyp. ... hypoparynx.
hypd. ... hypodermis.
int.s. ... internal sac.
i.o.l. ... median impressed line.
J. ... axillary area.
la. ... lacinia.
lb.p. ... labial palp.
lg. ... ligula.
l.lp. ... left lip.
M. ... media.
md. ... madible.
med.fr. ... median foramen.
med.or. ... median orifice.
med.str. ... median strut.
m.es. ... median epicranial suture.
mn. ... mentum.
mx.p. ... maxillary palp.
no. ... notaulices.
ob.s. ... oblique suture.
p. ... first costal.
pal. ... genital palp.
p.cl. ... post-clypeus.
ped. ... pedicel.
per. ... peritreme.
pf. ... palpifer.
pgr. ... palpiger.
pgm. ... convex process of post-clypeus.
p.m. ... prementum.
pn. ... pronotum.

p.o. ... post-occiput.
p.o.s. ... post-occipital suture.
post.l. ... postero-lateral portion of scutum
prth. ... prostheca.
p.s. ... pleural suture,
psc.2. ... meso-prescutum.
psc.3. ... meta-prescutum.
pscl.3. ... postscutellum.
ps.pl. ... pseudopleuron.
pt. ... posterior tentorial pit.
R. ... stigmatal area in Fig. 19 and Radius in Fig. 17.
r. ... radial cross vein.
rdg. ... ridge of furca.
rect. ... rectum.
r.lp. .. right lip.
r.m. ... radio-medial cross vein.
R.r. ... radial recurrent.
Rs. ... radial sector.
S. ... central.
s.8. ... eighth sternum.
sc.2. ... mesco-scutum.
sc3. ... metascutum.
sca. ... scape.
scl.2. ... meso-scutellum.
scl.3. ... meta-scutellum.
sm. ... submentum.
sn. ... subnotum.
s.pat. ... sensory patches.
sp.gas. ... spiculum gastrale.
st. ... stipes.
stl. ... sternellum (furca sternum).
7T-9T. ... seventh tergum to ninth tergum.
tar. ... tarsus.
tib. ... tibia.
tor. ... tormæ.
tr. ... trochanter.
tr.dp. ... triangular depression.
tract.pl. ... tractor plate.
trn. ... trochantin.
tr.r. .. transverse chitinous rod.
ut. ... uterus or vagina.
v. ... vertex.
v.s. ... scuto-scutellar or V-shaped suture.
w. ... oblong.

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A CONTRIBUTION TO THE MORPHOLOGY AND LIFE-HISTORY OF *SARGASSUM TENERRIMUM* J. Ag.[1]

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Introduction

No contribution has yet been made towards the life-history of Indian *Sargassums* in spite of their relative abundance. The genus is one of the most conspicuous and common of the sea-weeds which one can see either washed ashore or growing attached to rocks below low-tide level along the east and west coasts of India.

In 1848 J. Agardh described several species of *Sargassum* from this country (*Species Algarum*, Vol. 1, *Algae Fucoides completes*, Lund 1848) and in the following year R. K. Greville gave an account of fourteen species ("Algæ Orientalis—Description of new species belonging to the Genus *Sargassum.*", *Ann. and Mag. Nat. Hist.*, Ser. 2, Vol. 3). Boergesen (1933) also listed six of these species from Bombay in 1933 and one species from Ceylon in 1936. They were: *Sargassum tenerrimum* J. Ag. (which he thinks synonymous with *S. Campbellianum* Greville), *S. cinerum* J. Ag. var. *barberifolia* Grun., *S. Wightii* (Grev. mscr.) J. Ag., *S. ilicifolium* (Turn) C. Ag., *S. plagiophyllum* (Mort) J. Ag., *S. myriocystum* J. Ag., *S. cristaefolium* C. Ag. Apart from a taxonomic description of Indian species of *Sargassum*, no attempt has been made to give a detailed account of any of them. At the suggestion of Professor Y. Bharadwaja, the present work[2] was, therefore, undertaken by the writer to fill up this gap.

Historical

While a fair amount of anatomical and cytological work has been done on other members of the Fucales, very little is known about *Sargassum*. Miss Simons (1906) studied the development of conceptacles and embryo in *Sargassum filipendula*, while Nienberg (1910) described the oogonium and

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[2] This is a portion of the work submitted in part fulfilment for the M.Sc. Degree of the Benares Hindu University in 1939.

sporeling development in *Sargassum linifolium*. The later work is largely from Japanese workers of whom Tahara (1909) and Kuneida (1928) contributed the most. The former author records definite periodicity in the liberation of oogonia of *Sargassum* corresponding to that in *Dictyota*, while the latter gives a thorough account of the cytological details in the oogenesis and spermatogenesis of *Sargassum Horneri*. The present paper, however, deals with *Sargassum tenerrimum* J. Ag.

Material and Methods

The material for this investigation was collected from Bombay in January 1939.[3] It was killed and fixed in formalin-acetic-alcohol. Other fixatives such as Land's formalin-alcohol, Dr. Lynds Jones' formalin-alcohol, and chromo-acetic solution were also tried and found useful on material collected lately by the writer from Vizagapatam. The usual paraffin method was followed in sectioning the material, sections being cut from 8–14 μ thick. Staining was done with Heidenhain's iron-alum hæmatoxylin and Delafield's hæmatoxylin. The latter, owing to its staining the young walls, proved more useful than the former.

Morphology

The plant under investigation agrees in taxonomic characters with the *Sargassum tenerrimum* described and figured by Boergesen (1933) and Setchell (1935). J. Agardh (1848) described the plant originally from the coast of Bombay under the present name, while Greville (1849) described the same as *Sargassum Campbelliamum*. Grunow (1916) later referred to this form as a variety of *S. tennerimum* under the name var. *campbellianum*.

The plant has a basal adhesive disc or hold-fast from which arises a short primary axis bearing several branches together. The axis is rounded, glabrous, scarcely 2 mm. across. The secondary branches fork repeatedly in their turn. "Leaves" are 2–6 cm. long and $\frac{1}{2}$ to $1\frac{1}{2}$ cm. wide, gradually decreasing in size from the base upwards. They are thin, translucent and linear-lanceolate, with toothed margin and rather indistinct midrib. Cryptostomata are freely scattered on the "leaves" and receptacles while they are rare on the axis. The receptacles are freely-branched structures being attached to the upper portion of the stipe of the leaf. They are androgynous, spinose and show various cases of transformation of the branches. Cases have been observed where a portion of the receptacular branch may be a

[3] I am indebted to Captain S. C. Dixit, M.A., M.Sc., formerly of Wilson College, Bombay, or the supply of the material.

leaf, or a bladder, or may be surmounted by a leafy expanse. The floats are either borne separately on short stalks or assoicated with the very much branched receptacle branches. They are spherical or egg-shaped, the tip being commonly terminated by a small leafy appendage. This description coincides more with that given by Boergesen than that by Setchell who describes under the same name a plant from Hong-Kong in which the "leaves" are much smaller.

Anatomy

Simons (1906) summarises the histological work done on the genus up to 1906. The work of Reinke (1876) on *S. Boryanum*, of Oltmanns (1889) on *S. linifolium* and *S. varians*, of Hansteen (1892) on *S. bacciferum*, and the investigations of S mons herself on *S. filipendula* show that the growth of the axis and lateral organs takes place by means of a single 3-sided apical cell. In the 'stem' three kinds of tissues are recognised:—the assimilatory system, constituted by the epidermal cells; the storage system which is between the assimilatory and the innermost third system called the conducting system. According to Hansteen, the cells of the three systems are communicating with each other by pores, but S mons (1906) could not see such pores in her material of *S. filipendula*. In spite of best efforts, the writer also could not see pores in *S. tennerimum*.

In *S. tennerimum* also, both in the main axis and the laterals, there is a three-sided apical cell which contributes along with its neighbouring cells to the differentiation of all subsequent systems. The apical cell (Figs. 1 A and B) is situated in a pit or cavity which is its own creation, formed due to the quick succession in the production of cells in a tangential plane and the consequent lack of space for the elbowing of the resulting cells. It cuts off cells parallel to the three sides and the base and thus maintains its identity throughout.

The cells of the above-mentioned three systems get differentiated at some distance from the apex. The transverse section of the stem distinctly shows the three regions, namely, assimilatory, storage and conducting. The outermost assimilatory system consists of compactly-placed cells with convex outer walls, covered by mucilage. They are rich in chromatophores and reserve material. The cells of this system maintain their meristematic activity throughout their life and always divide at right angles to the surface. They are also responsible for the development of male and female conceptacles and cryptostomata. The cells of the second system, which also possess reserve material, are larger and possess wavy walls. They enclose many intercellular spaces. The cells of the conducting system are elongated,

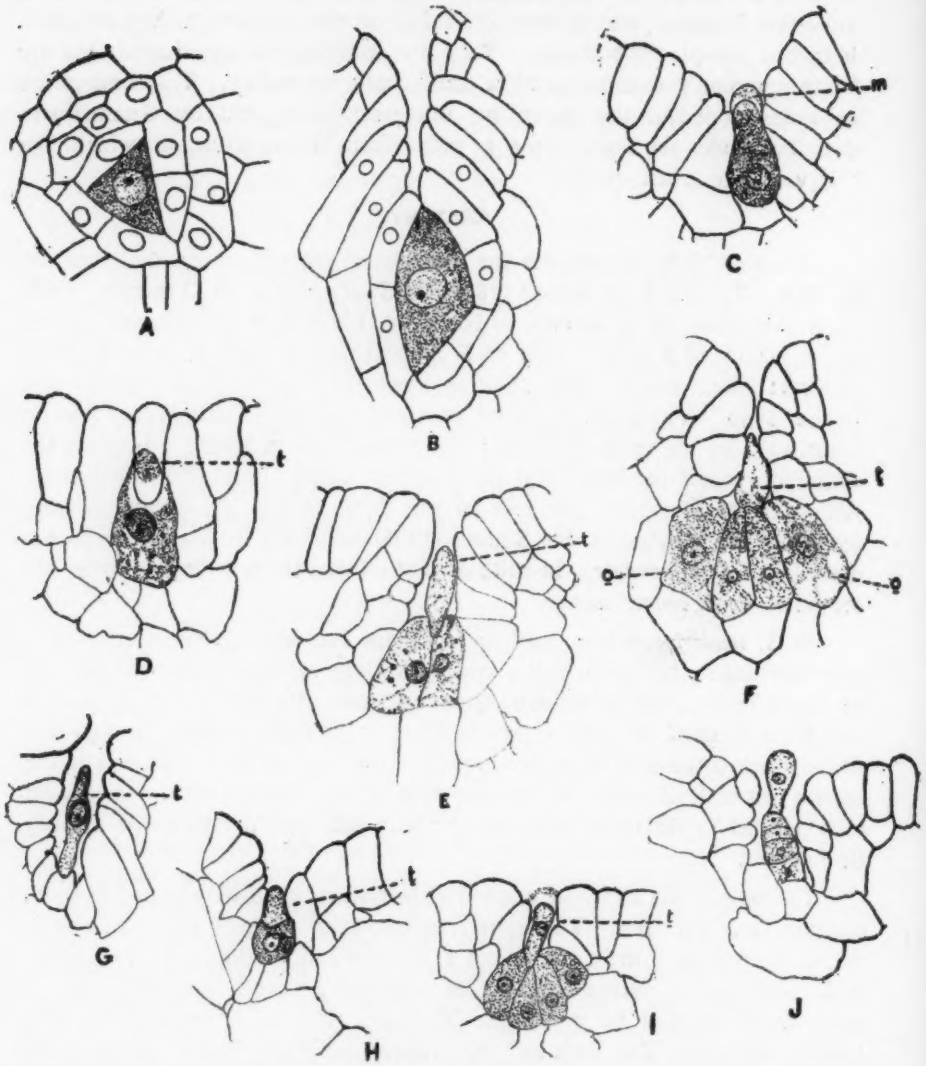

FIG. 1. *A*—transverse and *B*—longitudinal section of growing point showing three-sided apical cell; *C–G*—transverse sections of receptacles showing initial stages in the development of conceptacles. *C*—showing conceptacle-initial; *D*—showing two-celled stage; *E*—showing three-celled stage; *F*—showing five-celled stage; *G*—showing tongue-cell; *H–J*—stages in the development of cryptostomata. *H*—showing two-celled stage; *I*—showing five-celled stage; *J*—showing tongue-cell of the same dividing transversely. *t*, tongue-cell; *o*, oogonium initial; *m*, mucilage. All × 1650.

compactly arranged and possess oblique cross-walls. They lack in reserve material.

In a transverse section of the leaf also three regions can be recognized. The thickness of the leaf decreases as we pass from the midrib to sides, this being due to the reduction in the number of layers of cells that compose the storage tissue.

Receptacles and Conceptacles

Contrary to what we find in *Fucus*, the function of reproduction is relegated to specialized lateral branch systems. This character is also found in other Sargassaceous plants, such as *Bifurcaria, Cystophyllum, Marginaria, Carpophyllum*, etc. The receptacles are usually bisexual, although unisexual female receptacles are also met with. The conceptacles may be separated from one another by many layers of cells, or there may be only a little tissue between them. Their ostioles can be seen a bit raised over the general surface of the receptacle.

Development of Male and Female Conceptacles

The developmental history of conceptacles given by Bower (1880) for six plants of *Fucaceae*, that the initial cell does not contribute to the formation of the conceptacle, was supported by subsequent workers like Valiante (1883) (in *Cystoseira*), Oltmanns (1889) (in *Halydris siliquosa, Himanthalia lorea* and *Ascophyllum modosum*), and Grauber (1896) (in *Seiroccus*), till Simons (1906) in her account of *Sargassum filipendula* contradicted this view. She was supported by Nienburg (1910).

In early stages, the genesis of male and female conceptacles in *S. tennerrimum* is identical, so that a generalised description will hold good for both. Each conceptacle owes its development to a single initial cell which can be distinguished in the apical region of a receptacle by its possessing flask-shaped appearance, bigger size and larger nucleus and by getting more deeply stained than the surrounding cells. This cell may be one of those that have recently been cut off, or an old epidermal cell which regains functional activity. The lower part of the initial cell enlarges and is surrounded by storage cells, while the upper part is enclosed by the epidermal cells (Fig. 1 C). Later, a wall divides it into an upper cell, called by Simons the ' tongue-cell ', and a more or less bowl-shaped lower cell (Fig. 1 D). But, this wall in the present form is not so much depressed as figured by Simons (1906) (*cf.*, Pl. X, Fig. 2) in *S. filipendula*. Divisions are confined to the lower cell only, giving rise to 3-celled (Fig. 1 E) stage before further development occurs (Fig. 2, A–D). The upper cell persists as such till a

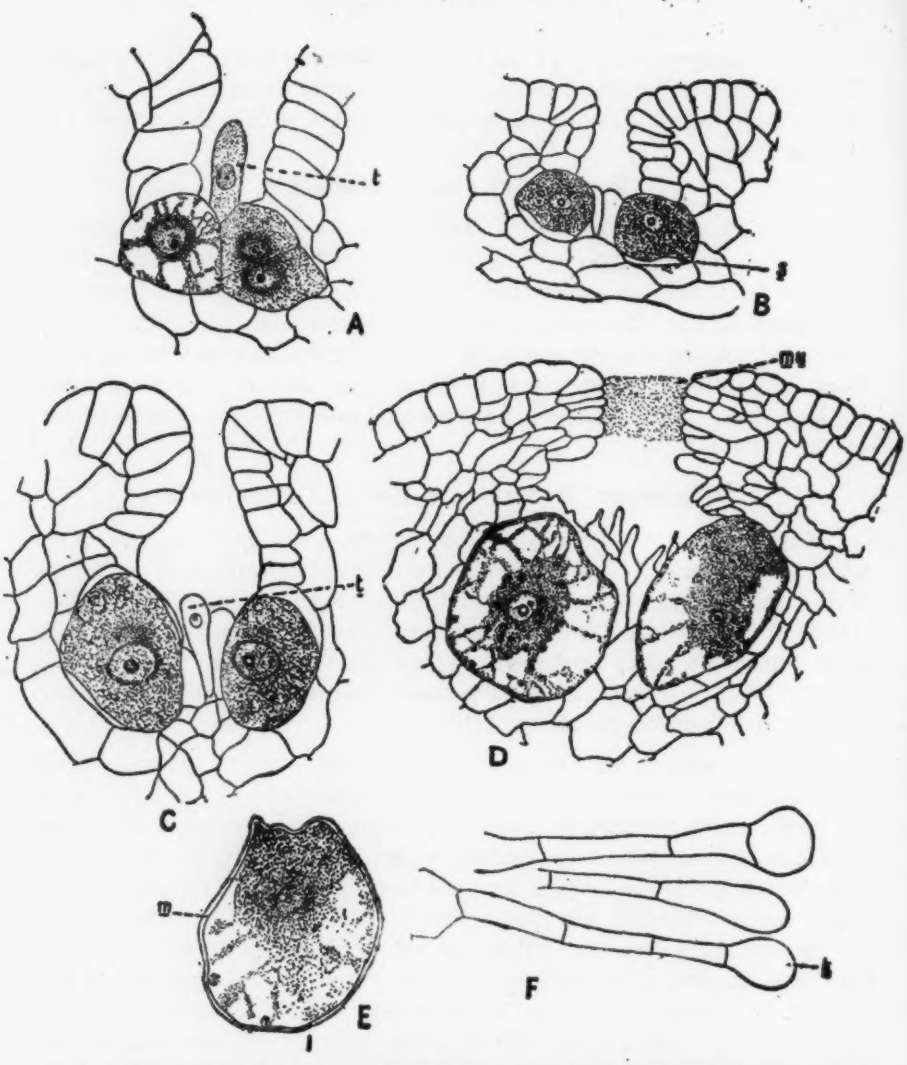

FIG. 2. *A*—Longitudinal section of female conceptacle showing transversely divided oogonium initial and the persisting tongue-cell; *B*—Later stage showing disintegrating stalk-cells of oogonia; *C*—still later stage, showing tongue-cell persisting; *D*—showing older oogonia with reserve food; *E*—oogonium with crescentic apical depression; *F*—paraphyses from a mature female conceptacle. *t*, tongue-cell; *s*, stalk-cell; *m*, mesochiton; *k*, knob-like swelling of paraphysis; *mu*, mucilage. *A* and *C* × 1650; *B* and *D* × 800; *E* × 300; *F* × 1330.

very late stage. The lower cells divide longitudinally, expand laterally and form the wall of the conceptacle.

In her paper on *S. filipendula*, Simons (1906) does not mention the exact stage at which the oogonium initials are laid down. But, in *S. tennerimum* they are laid down at a very early stage of the conceptacle (5 or 7-celled stage) (Fig. 1, F). Usually two to four (rarely up to six) oogonium-initials are laid down in each conceptacle. The rest of the wall-cells contribute to the formation of paraphyses which are unbranched filaments possessing elongated cells, the terminal cell being knob-like or club-shaped (Fig. 2, F). The paraphyses do not project beyond the ostiole.

The development of male conceptacle up to three or five-celled stage is similar, but further divisions are more rapid, giving rise to the cavity of the conceptacle lined by the potential antheridial mother cells. Here the paraphyses, unlike those of the oogonial conceptacles, are proliferations from the stalks of the antheridia. They may in some cases arise from the wall-cells also, but they are not multicellular structures (Fig. 3, G and H).

Cryptostomata

The development of the cryptostomata can also be traced to a single flask-shaped initial. Their early stages of development are similar to those of the male and female conceptacles. The tongue-cell, however, is generally seen to develop into a filamentous structure (Fig. 1, J) without undergoing disintegration as it does in the conceptacles. The cavity of a cryptostoma does not widen much. The paraphyses develop from wall-cells at a very early stage and grow throughout their life by means of a basal meristematic cell (Fig. 3, B, *b*), a type of basipetal growth which is taken to be the surviving remnant of the original extensive ' trichothallic ' growth, so commonly seen at present in the lower members of the Brown Algæ. The paraphyses in the mature cryptostomata project out freely (Fig. 3, B), and secrete mucilage

The writer has observed oogonium initials in two cases of cryptostomata of *S. tennerrimum* (Fig. 3, C) and this fact substantiates the view held by Bower (1880) and supported by other workers that the cryptostomata represent degenerate and non-functional conceptacles.

Oogonium[3]

As mentioned already, the oogonium initial is laid down very early in the development of the conceptacle (Fig. 1, F). This cell divides into two

[3] The term oogonium is retained to denote oogonial contents also as a matter of convenience, though it does not directly function as the female gamete as in other algae, but only after undergoing reduction division.

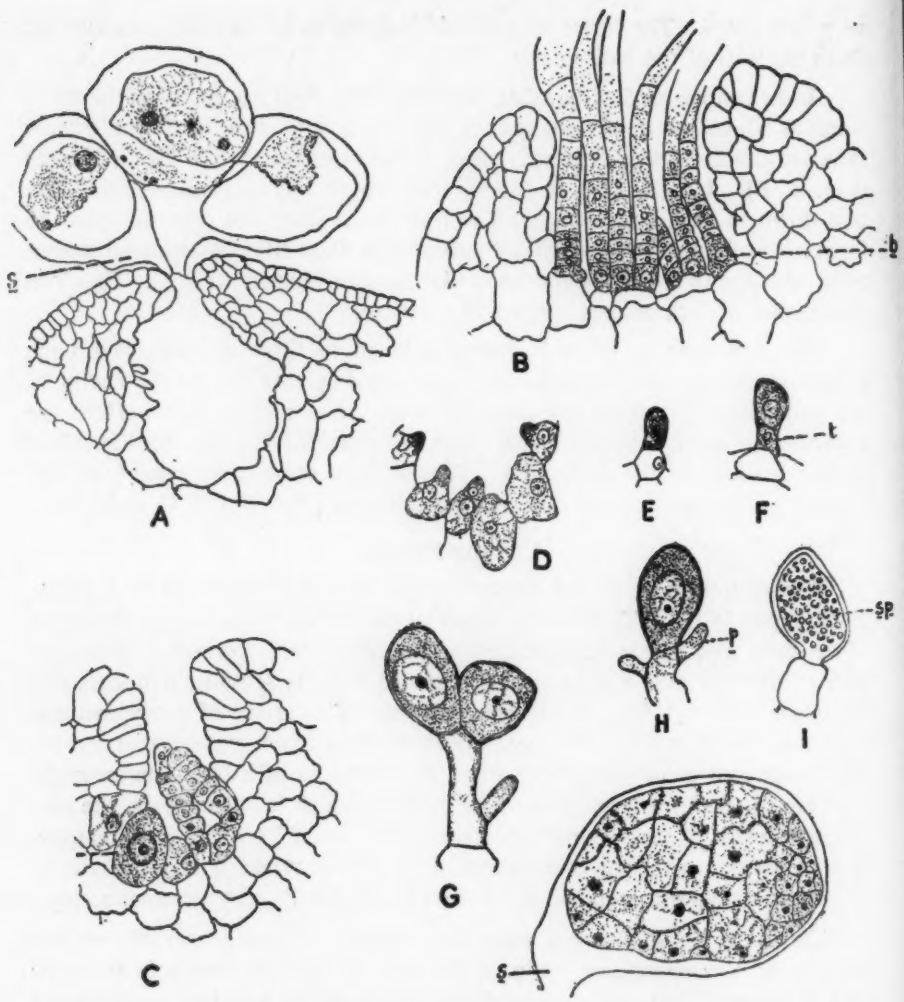

FIG. 3. *A*—A female receptacle with the liberated oogonia attached to the conceptacle by mean of mucilage-stalks ; *B*—mature cryptostoma from a receptacle, in longitudinal section with projecting paraphyses ; *C*—an exceptional case of cryptostoma showing oogonium. *D–I* ; developmental stages of Antheridium. *D*—Antheridial mother cells, one divided transversely ; *E*—Antheridial mother cell divided into two ; *F*—later stage of *E*, showing upper cell divided transversely into stalk-cell and antheridium proper ; *G* and *H*—mature antheridia with resting nuclei and unicellular paraphyses ; *I*—mature antheridium with sperms ; *J*—an advanced stage of embryo (reconstructed) ; *s*, mucilage-stalk ; *b*, basal meristematic cell of paraphysis ; *o*, oogonium ; *t*, stalk-cell of antheridium ; *p*, paraphysis of antheridium ; *sp*, sperms. *A* and *J* × 300 ; *B* × 800 ; *G* × 1330 ; the rest × 1650.

cells of equal size (Fig. 2, A). The lower is called the stalk cell, and the upper one, after enlarging, forms the oogonium. At a later stage the stalk cell is usually seen oppressed between the wall-cells and the enlarging oogonium (Fig. 2, B). Thus, unlike *Fucus*, the mature oogonium has no stalk cell. The oogonium enlarges and stores a lot of food material. Its nucleus, which also enlarges to some extent possesses a very prominent nucleolus and a little of chromatin material.

The wall of the oogonium differentiates into ' exochiton ', ' mesochiton ' and ' endochiton '. Later, the exochiton ruptures irregularly and the mesochiton becomes mucilagenous and forms the stalk by which the detached oogonia subsequently attach themselves to the mother plant. The development of mucilage does not appear to be localised to form a gelatinous cap, but mucilage occurs more or less all round the oogonium, thus differing from the observations made by Kunieda (1928) in *S. Horneri*, by Delf (1935) in *Bifurcaria brassiciformis* and by Dawson (1941) in *Cystoseira fæniculacea*. In the mature oogonium there is generally an apical crescentic depression with raised edges (Fig. 2, E).

Liberation of Oogonia

After the publication of Lloyd Williams' (1905) account of the periodicity in the liberation of sexual cells of *Dictyota dichotoma*, there appeared a paper by Tahara (1909) describing a corresponding periodicity in the liberation of oogonia in *Sargassum Horneri* and *S. enerve*, the time of liberation being three days in the former and one day in the latter after full moon. In *S. tennerimum* also the similarity in the developmental stages of individual groups of attached oogonia and embryos at a particular region of a receptacle is suggestive of a corresponding periodicity in the liberation of female sexual cells.

The actual mechanism involved in the liberation of oogonia is not well understood. Contraction of the tissues surrounding the conceptacle and the consequent gaping apart of the ostioles have been invoked as partial explanations (Farmer and Williams, 1898). Tahara (1913) has shown that in *Sargassum* tidal fluctuations influence extrusion of oogonia. Delf (1935), as reported by Dawson[4]) suggests that intensity and quality of illumination as well as hydrostatic pressure of the water are responsible for the extrusion of oogonia. Schreiber (1930) demonstrated in *Fucus* the liberation of oogonia by bringing about changes in turgor pressure of cells of conceptacle with the

[4] The observation of Delf referred to by Dawson in the former's paper published in 1935 could not be found by the writer.

help of solutes. As Dawson (1940) puts it " various factors, internal and external, would combine to raise the pressure in the conceptacle and force the oospheres out *via* the already slippery ostiole".

In her paper Delf (1935) reports her observations on the nature of liberation of oospheres of *Bifurcaria brassiciformis* and allied genera. After a careful study of several Fucaceous plants, she comes to the conclusion that the secondary attachment of oogonia by means of mucilagenous stalks formed by mesochiton are seen only in forms possessing unisexual conceptacles and this facilitates cross-fertilisation. But Dawson (1941) from a comparison of more than 30 members belonging to the Fucales refutes this statement, as mesochitonous stalks are met with in bisexual types also.

Simons mentioned that the nucleus of the oogonium in *S. filipendula* directly functions as the nucleus of the egg. But the investigations of Nienburg (1910) on *S. linifolium* and that of Kunieda on *S. Horneri* have shown conclusively that three divisions take place in the oogonium leading to the formation of eight nuclei. of which seven degenerate, while the eighth one enlarges and functions as the gamete nucleus.

There has been considerable difference of opinion regarding the stage at which the oogonia are liberated. Tahara (1913) states that the liberation of eggs takes place at the two-nucleate stage, this condition having been reached by the degeneration of six of the eighth nuclei. In a later paper Tahara and Shimatomai (1926) interpreted the binucleate condition of the oogonium as a result of the first division of the fertilized nucleus occurring simultaneously with the degeneration of the seven sister nuclei. Kunieda (1928) states that the nuclear divisions in *S. Horneri* take place while the oogonium is still attached to the conceptacle. In the Indian form, however, the writer has not been able to observe any case in which the nucleus of the oogonium underwent any division, while the latter was still attached to the wall of the conceptacle (Fig. 2 D). In the case of such oogonia as are secondarily attached to the conceptacle after having been released from the latter, eight nuclei were distinctly made out. This observation is in agreement with that of Oltmanns in a species of *Sargassum*, where he says that the nuclear division takes place after the liberation of the oogonia from the conceptacles.

That all the eight nuclei of the oogonium are potential gametes is shown by the behaviour of oospheres in *S. Horneri, S. enerve* (Tahara, 1913; Tahara and Shimotomai, 1926) and in *S. tortile* (Abe, 1938) where one peripheral nucleus may be fertilized in the presence of seven other equally vigorous gametes. Tahara (1927) could induce parthenogenesis in several

oospheres in an oogonium. Abe (1938) noticed that oocasionally two oospheres may be fertilized. The writer's observation of eight nuclei in the secondarily-attached oogonia also suggests that all the nuclei retain potentialities for fertilization and that the rest of the nuclei degenerate, when one nucleus gets fertilized. Thus, in the whole genus *Sargassum*, the oogonium should be taken to produce a single egg normally, although it contains eight potential oospheres.

Antheridia

The antheridia develop from the wall-cells of the male conceptacle. A cell puts forth a papilla-like projection into which large amount of protoplasm passes. The papilla is then cut off by a basal wall (Fig. 3, D–I). The lower cell remains as the wall-cell of the conceptacle, while the upper one *i.e.*, the papilla cell, divides into two, the lower of these forming a stalk cell and the upper enlarging and functioning as the antheridium. The antheridium has a big nucleus with a prominent nucleolus and a little amount of chromatin material. Each is associated with generally one-celled (rarely two-) paraphysis developed from the antheridial stalk-cell (Fig. 3, G). The mature antheridium (Fig. 3, I) has a thick mucilagenous wall.

Cytological details have not been worked out. Kunieda (1928) gives the haploid chromosome number in *S. Horneri* as sixteen. He also mentions that sixty-four sperms are formed in each antheridium and that each sperm is pear-shaped with two long cillia. In *S. tenerrimum* also sixty-four sperms have been observed (Fig. 3, I). It is interesting to note that even at a stage when we get young sporelings attached to the receptacles we still find such antheridia as have a primary resting nucleus, showing thereby that the receptacles are distinctly protogynous.

Embryo

Early stages in the development of embryo could not be observed. Mature embryos were met with plentifully. The sporeling which is found attached to the mother plant is spherical or ellipsoidal. The cells at one end are smaller than those at the other (Fig. 3, J), the former presumably giving rise to rhizoids later on. There appears no relation to gravity in the formation of rhizoidal cells.

Summary

1. The plant *Sargassum tenerrimum* J. Ag. has been studied. It is a monoecious species showing male and female conceptacles in the same receptacle.

2. Growth takes place by means of a single 3-sided apical cell.

3. The conceptacles and cryptostomata have been traced to single flask-shaped initial cell. Tongue cell in conceptacles does not divide transversely, but perishes after some time.

4. Oogonium initials are laid down at an early stage in the development of the conceptacle.

5. No mucilage cap is formed, as the mesochiton is uniformly thick all round.

6. The oogonium undergoes maturation-division only after the extrusion from the conceptacle. Only one egg is formed in an oogonium.

7. There is periodicity in the liberation of oogonia as indicated by the similarity in development of attached oogonia and sporelings at a particular region of the receptacle.

8. An antheridium produces sixty-four sperms.

9. Embryo produces small rhizoidal cells at its one end.

10. Except for a few differences, the developmental history agrees with that worked out by Simons in *S. filipendula*.

In conclusion, I have much pleasure in expressing my indebtedness to Professor Y. Bharadwaja for his kind guidance and criticism throughout the course of the investigation.

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THE RELATION OF PERMEABILITY TO ADAPTATION IN UNSTRIATED MUSCLE

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Danielli (1941) has explained accommodation in nerve produced by calcium as due to increase in permeability. Such an explanation would explain adaptation in unstriated muscle (Singh, 1944 *a*). In the present paper some facts are presented which harmonise with such a view.

Results

When unstriated muscle is stimulated continuously with alternating current, the muscle may be completely or incompletely tetanised, or relax during the passage of the current (Figs. 1 *a, b, c*). This suggests that if the

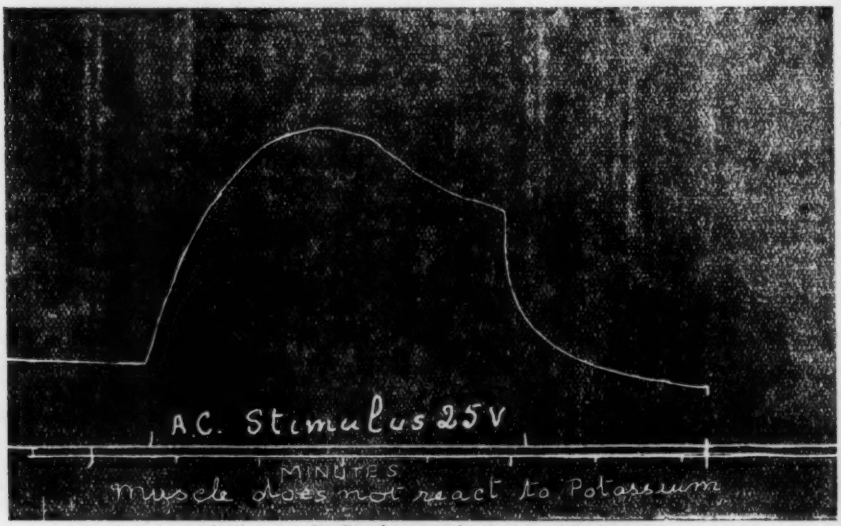

Fig. 1 *a*. *Mytilus* muscle. Continuous stimulation with alternating current

ions had accumulated on one side of the membrane, they had leaked through the membrane. As increase in the osmotic pressure of the saline produces identical contractions, or as the contraction is increased by increase in osmotic

pressure of the saline, the ions accumulate inside the muscle membrane, and the resulting inexcitability is probably due to their leakage through the

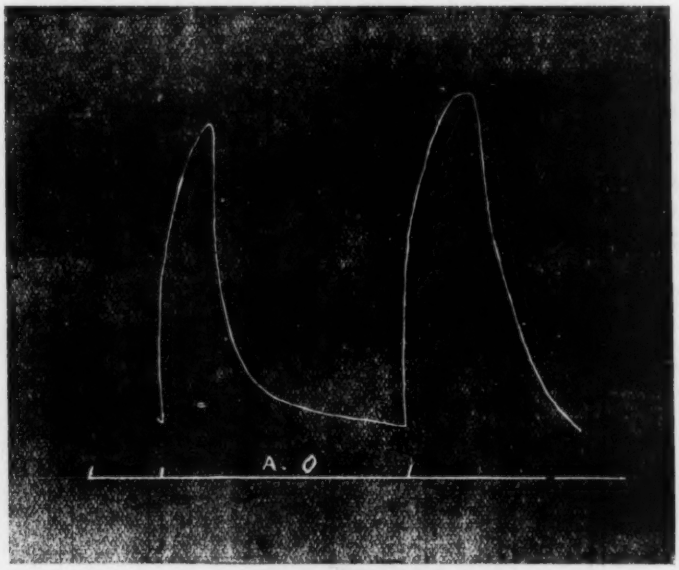

FIG. 1 *b*. Same as Fig. 1 *a*.

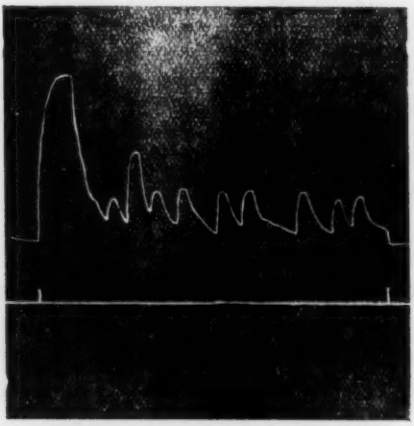

FIG. 1 *c*. Same as Fig. 1 *a*.

membrane, and the accumulation on the other side just as the inexcitability of the freshly dissected frog skeletal muscle (Duliere and Horton, 1929).

The above conclusion is supported by the following facts:—

(1) On cessation of the current, a contraction occurs, which has the properties of contractions produced by addition of ions outside the fibres.

(2) Substances which increase the permeability of the muscle, increase the subsidence of the tension and the off-contracture. Thus the latter is increased in the absence of the calcium and if the chloride of the saline is replaced by bromide, iodide, nitrate, thiocyanate and cyanide.

(3) During stimulation there is gain of sodium, which implies leakage of potassium (Singh, 1939). Thiocyanate and absence of calcium increases the leakage of potassium (Gokhale and Singh).

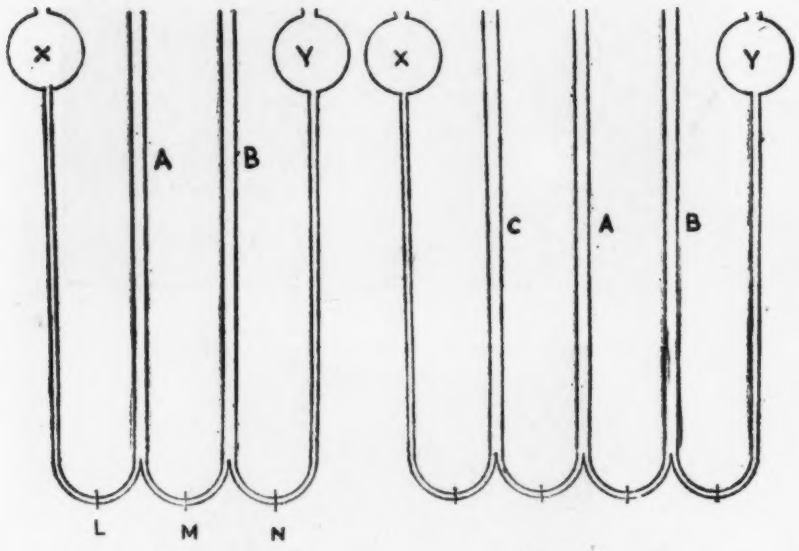

FIG. 2*a* FIG. 2*b*

The above phenomena can be represented by a simple hydrostatic model (Fig. 2*a*). It consists of a U-tube, with limbs A and B; each limb can be filled with mercury by means of reservoirs X and Y respectively. Excitation is presumed to occur when there is difference in levels of fluid in the two limbs A and B. The limb B represents the interior of the muscle fibres and the limb A, the exterior, L, M and N are variable constrictions. Raising of X implies additions of ions outside the fibres, and that of B stimulates excitation by electric current or increase in osmotic pressure of the saline producing an increase in the concentration of ions inside the fibres.

If X or Y are raised slowly then no contraction occurs, depending upon the permeability of M. The subsidence of the excitation process by electric current would be simulated by subsequent lowering of Y. This would result in a contraction similar to that produced by raising of X.

The occurrence of the off-contraction would be favoured by (i) increase of permeability, permitting leakage of ions. Thus it is favoured by the absence of calcium, and by bromide, nitrate, iodide, thiocyanate and cyanide, (ii) at the end of the stimulation, by decrease of permeability. Thus it is occasionally favoured by calcium. Excitation will be abolished if the permeability is too much increased.

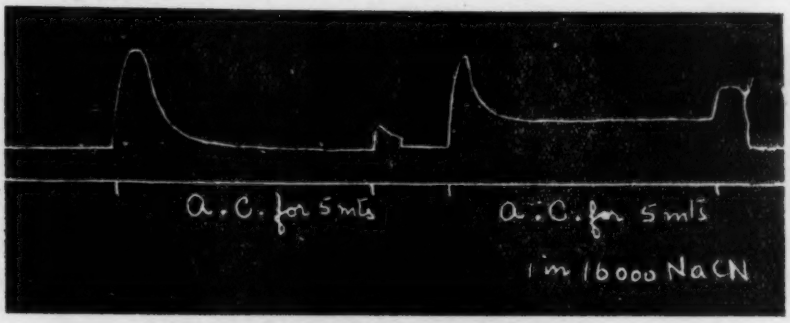

FIG. 3*a*. Same as Fig. 1 *a*. Effect of cyanide

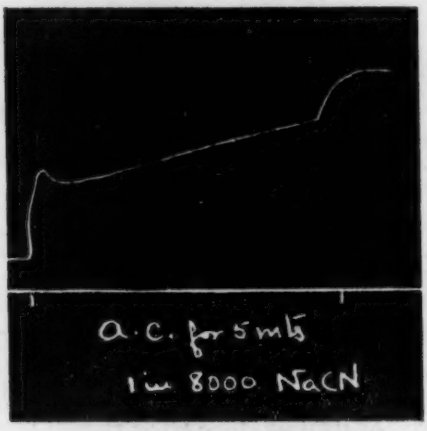

FIG. 3 *b*. Same as in Fig. 1 *a*

In the above model, an increase of permeability should lessen the excitability to electrical current, and to potassium. But the excitability to

potassium is oppositely affected. When *Mytilus* muscle is stimulated continuously with alternating current, a second contraction may occur (Fig. 3 *a, b*), which has the properties of the potassium contraction. Such curves resemble those in the striated muscle (Briscoe, 1938). In the model of Fig. 2, such a process would be simulated by raising of X, during raising of Y, so that the effect of Y may be neutralised or exceeded by that of X.

The above phenomena can be reproduced in the model by addition of another limb C on the side of X (Fig. 2*b*). C will represent the intercellular space instead of A and A a zone between B and C. With increase of permeability, ions will leak out into A both from B and C. With great increase in permeability the leakage of C may entirely mask the leakage from B, so that alternating current will produce a tonic contraction from the outset (Fig. 4).

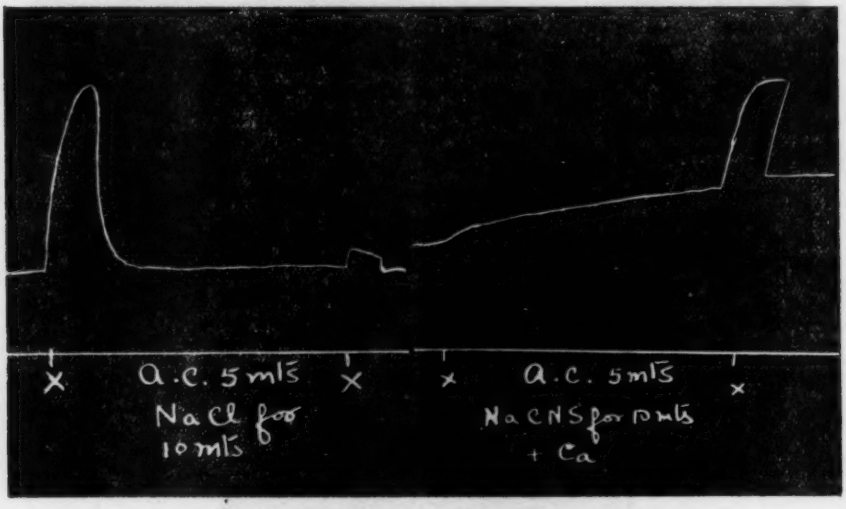

FIG. 4. Same as Fig. 1 *a*. Effect of the thiocyanate

The initial contraction has disappeared, but a tonic contraction has developed. The disappearance of the initial contraction and development of the tonic contraction can be traced by successive replacing the chloride of the saline with bromide, nitrate, iodide, and thiocyanate respectively (Singh, 1945).

At the end of the stimulation there will be an off-contracture due to leakage of the ions not only from B but also from C. Increase in permeability, therefore, will increase the excitability to chemical and diminish that to electrical stimulation. Adaptation to electric current would thus be due to two causes. As mentioned previously, the movement of the ions are not only conditioned by permeability changes but also by colloidal affinity (Singh, 1944 *b*).

Permeability has probably similar effect on chemical stimulation (Gokhale and Singh, 1945). It is possible however that adaptation may also be produced by the development of opposite excitatory states, which will produce a contraction of the opposite kind on cessation of stimulation.

Summary

A hydrostatic model is described to explain adaptation in unstriated muscle.

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COMPARATIVE PHYSIOLOGY OF UNSTRIATED CARDIAC, AND STRIATED MUSCLES

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Unstriated, cardiac and striated muscles differ structurally and functionally in many ways, yet there is an essential similarity in their responses. The question arises whether the differences are quantitative or qualitative. Does any one of the muscles possess some mechanism, which is not possessed by the other two, or if any special quality significant of one variety is merely held in abeyance in the other two. Thus it is well known that the chief property of cardiac muscle is rhythmicity, but under appropriate conditions, both the striated as well as unstriated muscles can be made to beat spontaneously. Isolated unstriated muscle shows tone; this is also exhibited by striated as well as cardiac muscle under certain conditions. The object of this paper is to correlate some of the phenomena presented by three kinds of muscles, and to see whether light can be thrown on the phenomena presented by unstriated muscle by a study of the other two. The author has repeated some of his experiments on unstriated muscle also on other kinds of muscle under identical conditions, so that the results may be comparable, as also the behaviour of the tissues is likely to show differences in the various parts of the world.

Experimental

The rectus abdominis of the frog was stimulated by Winton's method, as this method has been mainly employed previously in stimulation of unstriated muscle. The muscle was stimulated by alternating current, 2 volts for 10 seconds every 10 minutes. The voltage had to be increased to 8 volts as the muscle rapidly fatigued after a few tetanisations. The frog gastrocnemius was stimulated by single make and break maximal induction shocks every 5 or 10 minutes, the break shock following the make shock an interval of 15 seconds. In this way, the electrical stimulation was comparable to chemical, as the latter was resorted to every 5 or 10 minutes. The contractions were recorded with an isotonic lever slightly loaded or isometrically.

The experiments were performed at room temperature (32–37° C.). To compare the results of electrical, nervous or chemical stimulation, the

gastrocnemius of the opposite limbs were dissected out; one was then stimulated electrically or through the nerve, and the other with potassium or acetylcholine. Sometimes the same muscle was stimulated through the nerve or by acetylcholine every 5 minutes alternately, so that the effects of the two might be comparable on the same muscle under identical conditions.

Results

Two Excitabilities

Unstriated muscle can be made inexcitable to electrical stimulation and hyperexcitable to potassium (Singh, 1938 *a*). In the heart muscle the spontaneous contractions can be abolished, while the response to potassium is retained or may even be increased (Burridge, 1911, 1921, 1923 *a*). These results have been confirmed, though much smaller concentrations of potassium (0·04 M KCl) were required to produce a powerful contraction. The heart muscle thus behaves like unstriated muscle in this respect.

In the striated muscle, I have not been able to find any significant difference between the responses to electrical stimulation and that to potassium, except in a few experiments. Burridge (1911) described the differential action of drugs on the response to electric current and potassium. I have tried the reagents which gave a differential action on *Mytilus* muscle, such as calcium, magnesium, the anions, chloride, bromide, nitrate, iodide, and the drugs adrenaline, acetylcholine, veratrine, strychnine, eserine, pilocarpine, curare (6 experiments each, but 8 in the case of adrenaline and curare). Curare used was an impure product (Merck).

Gelhorn and Northrup (1932) found in the frog striated muscle, that the response to potassium increased after indirect stimulation. In the present experiments (2 out of 8) it was found that curare (saturated solution in the Ringer) in the frog gastrocnemius muscle, increased the response to potassium, while diminished that to electrical stimulation. The response to acetylcholine as well as that to indirct stimulation was decreased (Fig. 1). These results are significant in view of the fact that heavy doses of curare increase the chronaxie (Lapique, 1906; Watt, 1924), and make the neurone more sensitive to potassium and insensitive otherwise (Brown and Feldberg, 1936). In the other 6 experiments, the response to potassium was also depressed.

Adrenaline, 1 in 10^6, in 2 out of 8 experiments increased the response to potassium, and decreased that to electrical stimulation (Fig. 2). In the remaining 6 experiments, the response to electrical stimulation was either slightly increased or unaffected. Increase in the osmotic pressure of the saline by addition of sodium chloride or sucrose to twice normal, increased

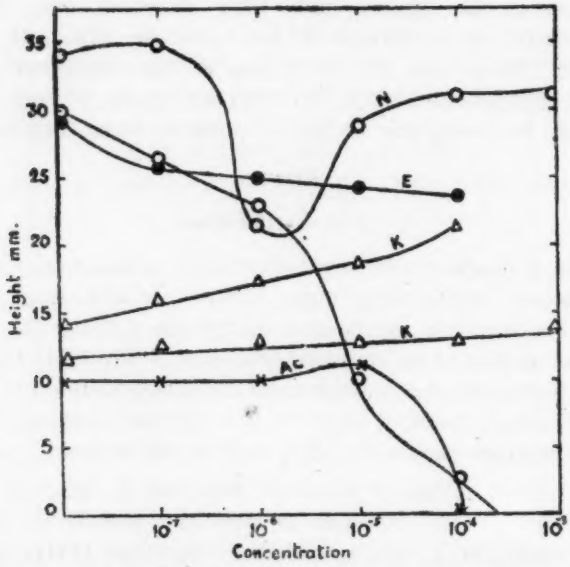

FIG. 1. Frog gastrocnemius. Effect of curare on the response to induction shocks (E), potassium (K), acetylcholine (A.C.) and nervous stimulation (N)

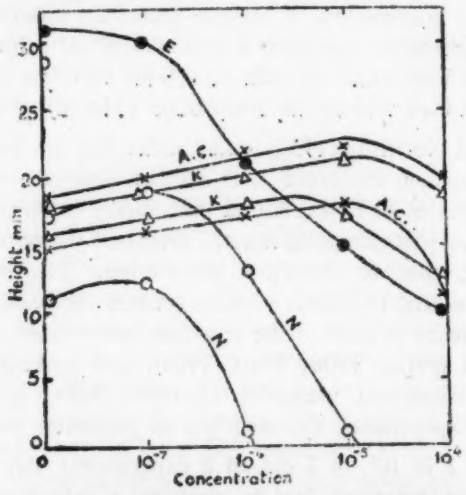

FIG. 2. Frog gastrocnemius. Effect of adrenaline

the response to potassium and decreased that to electrical stimulation in 2 out of 6 experiments (Fig. 3). In the remaining 4, the response to potassium was also depressed; in 2 experiments the response to electrical stimulation

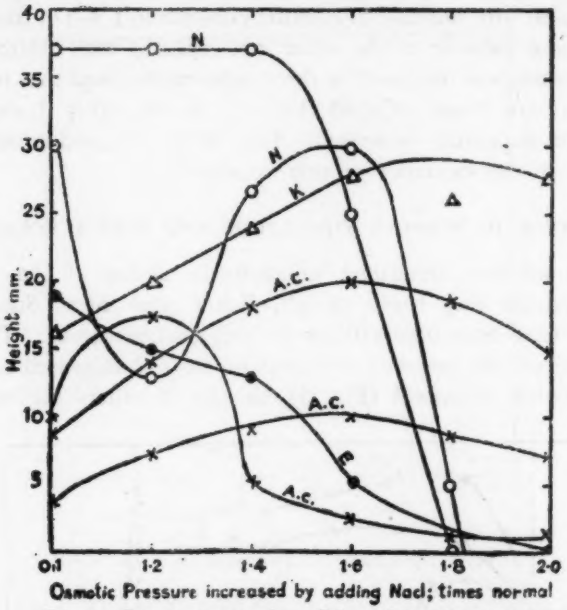

FIG. 3. Frog gastrocnemius. Effect of osmotic pressure

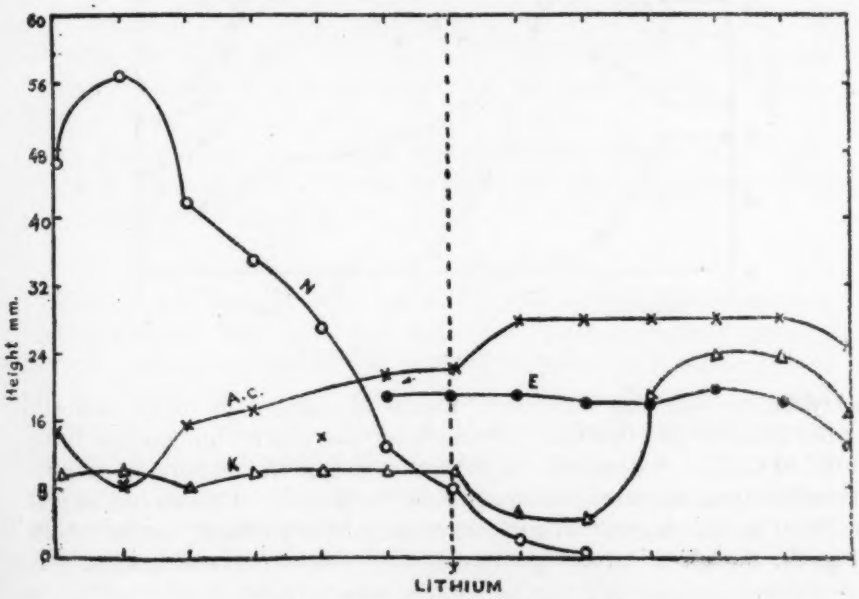

FIG. 4. Frog gastrocnemius. Effect of lithium

was also increased with increase of osmotic pressure to 1·4–1·6 times normal. If 60% of sodium chloride of the saline was replaced with lithium, the response to potassium was increased in three experiments, and that to electrical stimulation was not much affected (Fig. 4). In the other 3 experiments, the response to potassium decreased. Thus in the striated muscle, there is some evidence of the existence of two excitabilities.

Response to Nervous Stimulation and Acetylcholine

Previously we have described antagonistic action of the vagus and acetylcholine on the frog heart (Singh, Sehra and Mrs. Singh, 1945). Similar results have been obtained on the frog gastrocnemius. Thus in the absence of calcium, the response to acetylcholine was increased and that to nervous stimulation decreased (Fig. 5), though in others the response to

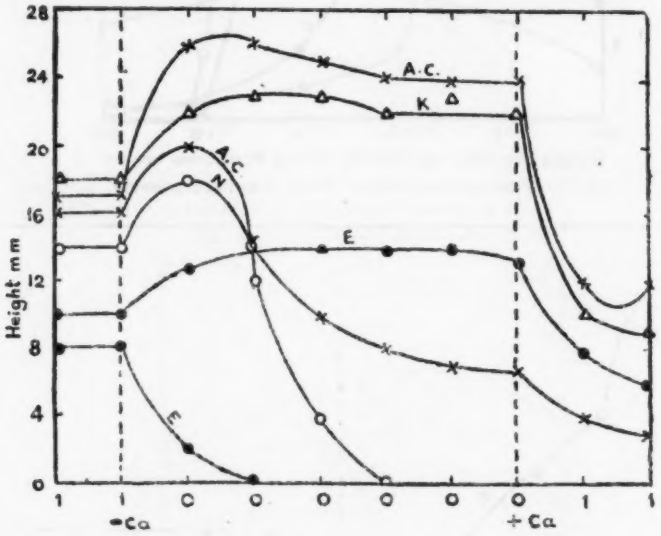

Fig. 5. Frog. gastrocnemius. Effect of calcium

acetylcholine was also depressed. Excess of calcium produced opposite results (Fig. 6), the optimum concentration for indirect stimulation being 0·002 M $CaCl_2$. Adrenaline 1 in 10^5 also depressed the response to nervous stimulation and increased that to acetylcholine (Fig. 2). Lithium had similar action (Fig. 4). Increase of osmotic pressure also produced similar results (Fig. 3), though in some experiments, the response to acetylcholine was epressed,

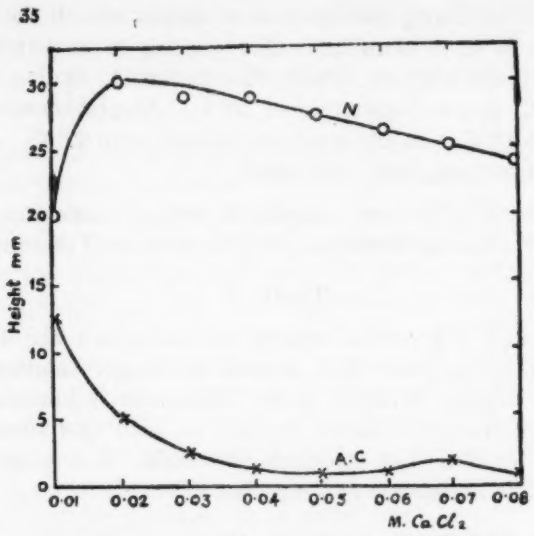

FIG. 6. Frog gastrocnemius. Effect of calcium

Then further it was sometimes noticed, that when the muscle was highly sensitive to nervous stimulation, it was relatively insensitive to acetylcholine, the concentration of the latter required to stimulate being 1 in 2,500. At other times, the muscle behaved in an opposite manner (Fig. 4), the muscle becoming inexcitable to nervous stimulation, and hypersensitive to acetylcholine.

The dog stomach preparations also sometimes behaved in a similar way. With repeated stimulation the response to vagus disappeared, while that to acetylcholine remained.

Effect of Temperature

While at Allahabad, I often wondered, how my gardener could work in the intense heat, that would have killed me. I found that the optimum temperature for the response of dog stomach preparations at Bombay to be 25° C. At Patna, where the temperature was 115° F. in the shade, the optimum temperature was 30° C. This difference is probably due to adaptation on the part of the animals to a higher temperature.

The frogs in India have to pass through extremes of seasonal variations of temperature. In Hyderabad, the frogs which were experimented upon had seen exceptionally hot summer and an exceptionally cold winter. The room temperature in our laboratory is often 37° C. The frog gastrocnemius

presented a most interesting phenomenon of adaptation. If the temperature of the saline was 30° C. at room temperature, raising of temperature to 35° C. at first depressed the response, which then increased, so that the isotonic response at 35° C. may be bigger than at 30° C. Adaptation was thus found to occur upto 35–37° C. In one muscle it occurred upto 40° C. At 42·5° C., the muscle practically becomes inexictable.

Similarly hearts which had adapted to higher temperature, would at first stop beating when the temperature was lowered, and then resume again.

Tonus

Both unstriated and cardiac muscles exhibit tone. The frog striated muscle here, when immersed and aerated in Ringer solution, develops tone (*cf.* Ringer tonus; Winton, 1930). This tone is inhibited by adrenaline and by increase in osmotic pressure, as with frog stomach. Time extension curves are like those of unstriated muscle. It is possible that this is a contracture due to increase of temperature.

When tone develops in unstriated muscle its excitability diminishes. After a contraction is produced by alternating current, nervous stimulation, potassium and acetylcholine, this tone diminishes for a brief period. It happens with gastrocnemius also. The contracture produced by acetylcholine is diminished by twitch. After preliminary inhibition following the twitch, the contracture re-develops. The same thing was found in the heart with potassium contracture (Singh, Sehra and Mrs. Singh); from this we concluded the presence of two excitabilities in the heart muscle. The above experiments show that this conclusion was wrong, the inhibition was due to the fact that every excitatory process is accompanied by an inhibitory one. As regards the veratrine effect it also becomes less and less marked if the muscle is made to give repeated contractions, but re-appears after a suitable period of rest.

In unstriated muscle, tonic contraction is due to some structural change, probably increase in viscosity, or to active contraction; the twitch affects the contraction as above, but with this difference that in the former, tension does not re-develop, but does so with tonic contraction. It thus appears that tone or viscosity are due to similar causes as both are antagonised by twitch. Some ionic change, therefores acts upon plain muscle to produce either tone or increase in viscosity. Striated muscle is comparatively much less susceptible than unstriated muscle in undergoing changes in viscosity. One kind of tone in the unstriated muscle is therefore identical with contracture in the striated muscle. This tone requires oxygen.

Whatever causes contracture in striated or unstriated muscle, causes slowing of relaxation in both. The special property of unstriated muscle leads to structural changes, so that energy is not required for maintenance of the contraction.

Contracture is antagonised by twitch; it depresses excitability in striated, cardiac and unstriated muscle. Thus if potassium, acetylcholine cause a contracture, further responses by heavier concentrations of these substances are depressed. Thus acetylcholine is depressant to acetylcholine. This shows that the contracture is produced by another agency, these substances setting up a chain reaction. This substance is present inside the fibres, as a depressant contracture and slowing of the relaxation occurs in isotonic sucrose, and as it is diminished by decrease of osmotic pressure (Singh, 1944 *a*), though there is evidence that sodium chloride of the saline may also be concerned (Singh, 1938 *b*, 1943). The above view is supported by experiments of Langley (1913) on striated muscle, and those of Gokhale and Singh (1945) on unstriated muscle; they found that the contracture may persist, though the stimulating substance is withdrawn.

Contracture also occurs which is synergistic to twitch in striated and unstriated muscle. Thus in frog rectus abdominis acetylcholine contracture may be synergistic to contractions produced by alternating current. In frog gastrocnemius and frog stomach lithium may produce similar results. This suggests that the contractile mechanism ultimately for tonic contractions and twitch is the same. But the differential action of certain substances on the viscosity and the contractile mechanism suggests that at least the slow relaxation is sarcoplasmic and contraction, the property of myosin in the sarcostyles. This is a modification of the view put forward by Botazzi.

Excitability to Chemical and Electrical Stimulation

Skeletal muscle is more excitable to electrical stimulation than unstriated muscle, the heart muscle showing an intermediate value but the excitability to chemical stimulation varies. I have not noted much difference between the sensitivity of striated and unstriated muscles to potassium. Sometimes I have found the frog stomach to be much less excitable to acetylcholine than the rectus abdominis or the gastrocnemius.

The heart muscle is least excitable to potassium and acetylcholine. The reasons for this are probably as follows:—Every stimulus produces both excitatory and inhibitory effects (adaptation). In the heart muscle the inhibitory process is probably more labile than in the other two muscles. This is shown by the fact that it reacts by intermittent contractions rather

than a continuous contraction to a constant stimulus. Calcium may convert complete tetanus into intermittent contractions in cardiac muscle (Singh, Sehra and Mrs. Singh, 1945 *a*, *b*). This is quite a common effect of calcium in unstriated muscle (Singh, 1938 *c*; 1942).

The depressant action of drugs and ions varies in the three kinds of muscle, there being a gradation from unstriated to cardiac and striated muscles. Thus large doses of adrenaline (1 in 10^5) are most depressant to frog stomach, less to frog heart and frog gastrocnemius. The anions bromide, iodide, nitrate and thiocyanate have similar actions. This is probably determined by the resistance of their membranes. The calcium concentration is greater in frog striated muscle than in unstriated muscle (Meigs and Ryan, 1912), though in other animals it is the opposite (Wilkins, 1934).

The resistance of the membranes is probably determined not by the concentration of calcium in the whole fibre, but that in the membrane itself and these may not run parallel. In this connection it is interesting to know that the electrical resistance of unstriated muscle is less than that of striated muscle (Singh and Mrs. Singh, 1944).

Acetylcholine and potassium are more depressant to the heart than unstriated or striated muscles. This anomaly is due to the fact that inhibition has to be distinguished from toxic action. In hearts, where acetylcholine has a stimulant action, large doses are required to produce depression. In such cases the question arises whether the depression due to these toxic doses is the same as the inhibition produced by small concentrations. As acetylcholine sometimes produces stimulation of frog heart in small doses and depression in large doses, is the action of acetylcholine purely stimulant? Vagus in these hearts have produced complete standstill. The question then arises whether the action of acetylcholine is antagonistic to that of the vagus or the concentration of acetylcholine secreted by the nerve is high, and is in more intimate contact with the muscle fibres.

Adaptation

The frog stomach and the gastrocnemius behave very differently when stimulated by alternating current. In the former adaptation is rapid and in the latter slow. Adaptation to acetylcholine also is similarly affected. If adaptation is due to calcium, then it shows that though the frog stomach contains less calcium than gastrocnemius, it is in a more labile form. Frog stomach is more resistant to the toxic action of potassium (Hober, 1927) than the gastrocnemius; this also suggests more labile calcium in the former, as calcium is antagonistic to the action of potassium.

Effect of Drugs and Ions

Effect of calcium.—The frog gastrocnemius resembles *Mytilus* muscle in that it contracts in the absence of calcium. In both, when calcium is excluded, there is hyperexcitability to potassium and acetylcholine, and this is followed by a depression in a few minutes. In the frog gastrocnemius, however, the excitability to electric current is raised, whilst that in the *Mytilus* muscle, is depressed. This is probably due to the fact that in frog gastrocnemius adaptation plays a major part (Singh, 1938 *d*). This is shown by the fact that the response to potassium and acetylcholine also increases. If contracture develops, then these responses are depressed.

The opposite kind of response was also noticed, that is the muscle became inexcitable to electrical and nervous stimulation, and hyperexcitable to potassium and acetylcholine. The response of the first kind could be changed into second by fatiguing the muscle by tetanising it with alternating current for 10 seconds every 10 minutes. When the steady state is reached, the muscle becomes inexcitable to electric current in the absence of calcium.

A fatigued striated muscle thus resembles unstriated muscle in that (i) it becomes inexcitable in the absence of calcium; (ii) its movements are sluggish; (iii) it may show contracture; (iv) it may be more sensitive to potassium; (v) threshold to electrical stimulation increases; and (vi) its sodium content may increase (Fenn and Cobb, 1934).

The frog heart resembles the stomach, in that its excitability diminishes in the absence of calcium. The heart muscle is thus nearer to unstriated muscle than to striated muscle in this and other properties.

After being deprived of calcium, the frog gastrocnemius, the guinea pig uterus, and the frog heart (Burridge, 1915) become more sensitive to it. This is due to adaptation.

Action of Sodium Chloride.—Replacement of part of the sodium chloride of the saline with sucrose (or glucose in the case of *Mytilus* muscle), decreases the response to electric current in frog stomach and gastrocnemius, *Mytilus* muscle and the spontaneous contractions in the frog heart, though the exactly opposite also happens. In *Mytilus* muscle, the response increases when it shows much tone and the increase is excitability is due to removal of excitatory action of sodium chloride which is antagonistic in the same way as is potassium chloride. In the frog heart and frog gastrocnemius it is due to removal of the inhibitory effect of sodium chloride. In *Mytilus* muscle, decrease in excitability to electric current occurs in sodium deficient solutions, when it does not show much tone; this is a common finding in the frog gastrocnemius, which does not show tone.

The response to potassium decreases in the *Mytilus* muscle and increases in the frog stomach in sodium deficient solutions for the above reasons. In the guinea pig uterus and *Mytilus* muscle, tone decreases. In the frog gastrocnemius also the response at first increases.

When 80% of the sodium chloride is replaced, the frog gastrocnemius may become hyperexcitable to potassium and less excitable to electric current or the two excitables may be identically affected.

Partial replacement of sodium chloride of the saline increases the inhibitory action of adrenaline and ammonium in frog stomach, the inhibitory action of adrenaline in guinea pig uterus, and that of acetylcholine in frog heart, if the contractions decrease in height. If the contractions increase in height, then the opposite happens.

When sodium chloride is completely excluded, then the frog stomach, frog heart, frog gastrocnemius and guinea pig uterus enter into contracture, and adrenaline loses its inhibitory action on the guinea pig uterus and the frog stomach and acetylcholine on the frog heart. Their excitatory action is also lost (Singh, 1945). Thus these drugs bring about their action probably through a chain reaction, by sensitising the muscle to other ions. The contracture in sodium chloride deficient solution shows that in all the three kinds of muscle, contraction is produced by a difference in concentration of ions on two sides of the muscle membrane.

In the electrolyte-free medium, the frog gastrocnemius becomes absolutely inexcitable, though Chao (1937) has described that toad muscle remains irritable for 6 hours in the pure sucrose solution. Some frog hearts may become hyper-irritable, in that the magnitude of contractions of ventricles and auricles, which beat independently, is increased; in others, there is complete inexcitability. Frog stomach also behaves similarly. The magnitude of the spontaneous contractions may be greatly increased, though in others they are completely suppressed. In some of the latter, a trace of potassium chloride (1 in 10^5) may restore them.

Effect of lithium chloride.—In frog stomach, guinea pig uterus, *Mytilus* muscle and frog gastrocnemius, some of the effects are those of sodium deficiency. Frog stomach contracts and becomes hyperexcitable to alternating current and potassium, owing to the fact that its inhibitory action is less than that of sodium. Similarly in the guinea pig uterus and *Mytilus* muscle, tone decreases, as its excitatory action is less than that of sodium. *Mytilus* muscle becomes hyperexcitable to potassium. In frog gastrocnemius, replacement of 60% of the sodium chloride of the saline renders the

muscle hyper-irritable to potassium and acetylcholine, less excitable to electric current and inexcitable to nervous stimulation.

The response of the frog gastrocnemius to potassium decreases if contracture develops, and that to electric current may increase. In *Mytilus* muscle the response to acetylcholine decreases or increases. The increase is due to the increased excitability to stimulations from without, and the decrease to removal of potentiating effect of sodium.

The varied responses mentioned above are due to the fact that the action of sodium chloride may be inhibitory or excitatory and that contracture diminishes the excitability.

Effect of ammonium.—Ammonium is depressant to the frog stomach, but increases the excitability of the gastrocnemius and the heart in small concentration (0·002 M NH_4Cl). Larger concentrations are depressant. In *Mytilus* muscle the excitability to alternating current is increased.

Effect of potassium.—0·02 M KCl stimulates frog stomach and gastrocnemius, but depresses the heart, though I have come across frog hearts in which the action was excitatory. The resistance of the frog stomach as well as the gastrocnemius to excess of potassium varies. All the three muscles exhibit adaptation to normal concentrations of potassium (potassium paradox). In guinea pig uterus, all concentrations of potassium may be inhibitory.

Effect of hydrogen-ion concentration.—The action of hydrogen ions may be excitatory or inhibitory in striated muscle and unstriated muscle. In all the three kinds of muscle, alkali increases the excitability. *Mytilus* muscle and guinea pig uterus show adaptation to hydrogen ions. In *Mytilus* muscle, sudden increase of hydrogen ions to pH 4·4 increases the excitability to potassium or cause contraction; slow increase has an opposite effect.

Effect of adrenaline.—In the *Mytilus* muscle, frog stomach and gastrocnemius small concentrations (1 in 10^7) increase the response to electric current, and the spontaneous contractions in the frog heart. In *Mytilus* muscle which are inexcitable to potassium and show little tone, larger contractions have a similar action, so also in the frog gastrocnemius which has no tone. In other *Mytilus* muscles and in frog gastrocnemius, adrenaline (1 in 10^8) decreases the response to electrical and nervous stimulation, and increase that to potassium and acetylcholine. Adrenaline has also a depressant action on frog heart and frog stomach. Thus like most substances adrenaline may have a depressant or excitatory action.

Effect of acetylcholine.—Acetylcholine may also have an excitatory or inhibitory action on the frog stomach, heart and gastrocnemius.

Effect of initial length.—Increase in the length of the fibres increases the excitability in all kinds of muscle, though the opposite has also been found in *Mytilus* muscle and frog heart. I have come across frog hearts which do not obey the Starling's law. This is probably due to stimulation of the intracardiac vagus, as the action of acetylcholine and that of vagus also increases with the initial lengths in these hearts; hearts which obey Starling's law behave oppositely.

Effect of osmotic pressure.—Increase of osmotic pressure diminishes tone. In the frog stomach and heart, increase of osmotic pressure to 1·4 times normal may increase or decrease the excitability; actual contraction may be caused. In the frog gastrocnemius, the response to potassium and acetylcholine increases. In *Mytilus* muscle it decreases; the response to electric current may increase or decrease.

The effect of osmotic pressure depends upon the fact that it increases the concentration of ions within the muscle fibres and thus may increase their excitatory or inhibitory action, and antagonises the excitatory and inhibitory action of the ions outside. This is shown by the fact that in the frog stomach, increase in osmotic pressure increases the response to potassium, but if the potassium contracture is induced, then increase of osmotic pressure dimiishes the contracture. In the first instance the inhibitory effect of sodium was antagonised, but when the contracture develops this is done by the potassium itself, so that increases in osmotic pressure now cause relaxation.

The effect of increase in osmotic pressure shows that the action of sodium chloride on the frog gastrocnemius is inhibitory ; that of calcium lack shows that it is excitatory, so it probably contains two antagonistic factors (Singh, 1942).

When tonicity of the saline is increased by adding sodium chloride, the response to electric current at first decreases and then increases in *Mytilus* muscle, frog stomach heart (spontaneous contractions) and the gastrocnemius. Burrdige (1922) has shown that the depression and increase in excitability are determined by independent factors, which is in agreement with my views that depression is due to the action of ions without, and the increase to increase in concentration of ions within the fibres the depressant action of sodium chloride being akin to that of potassium.

Excitation and Inhibition

Both in unstriated and cardiac muscles excitation and inhibition may be affected identically. Thus calcium antagonises the stimulant as well as the depressant action of pilocarpine (Burridge, 1923 *b*), and those of acetylcholine

(Burridge, 1935) on the frog heart. This shows that they are dependent upon some common property of the muscles (Singh, 1945), inhibition being a result of excitation. In such instances, increase in permeability will increase excitation, as well as inhibition. If the inhibitory agent is very labile, then the causative excitatory action may be masked, and only inhibition produced. This probably accounts for the inhibitory action of acetylcholine on the heart muscle. If it is possible to antagonise the excitatory process, then all excitatory substances should produce inhibition. If it is possible to antagonise the inhibitory process, then all inhibitory substances should produce excitation. This dual action is latent in all excitatory or inhibitory substances, and by suitable alterations in the environment either action should be produced.

The Motor End Plate

A most interesting result is that substances which produce curarisation in the frog nerve muscle preparation abolish the response of *Mytilus* muscle to electrical stimulation. These are lack of calcium, curare, excess of strychnine, veratrine, etc. Ammonium also produces similar results (Ing and Wright, 1931; Singh, 1938 *a*). This would suggest that either the motor end plate is similar to *Mytilus* muscle in the constitution of its excitability or that the muscle can only be excited through its nerves. The latter is most unlikely, as curarisation is not known in plain muscle.

It therefore appears that unstriated muscle can be directly excited by nerves, but not striated muscle. For the transmission of the impulse, a structure like that of plain muscle must first be excited which will then conduct the excitation to the striated muscle. This explains why motor end plates are not necessary for the excitation of unstriated muscle.

The above conclusion would be strange, because excitation will have to pass from a more excitable to a less excitable tissue through a least excitable one. But then unstriated muscle is known to be very sensitive to chemical stimulation, so that a chemical transmission from nerve to the motor end plate would be very proper. From the motor end plate to the muscle, the transmission would be electrical, so that the whole transmission from nerve to muscle would be electrochemical in striated muscle, and chemical in unstriated and cardiac muscle.

Nerve endings in unstriated muscle may end within the cell or without. This provides a twofold method of nervous action, that is, excitation from within and that from without (Singh, 1944 *b*).

Summary

1. Unstriated, cardiac and striated muscles show differential action of substances on the excitability to electrical stimulation and potassium.

2. The response to nervous stimulation and acetylcholine may be similarly or oppositely affected.

3. Muscle shows adaptation to temperature.

4. Tonus may be shown by three kinds of muscle; one kind of tone in unstriated muscle is similar to the contracture of striated muscle.

5. In all the three kinds of muscle, twitch is antagonistic to contracture; a synergistic contracture also occurs.

6. In some instances, there is a gradation in the properties of unstriated, cardiac and striated muscles.

7. Adaptation to electric current is more rapid in unstriated than in striated muscle.

8. Many drugs and ions act similarly on the three kinds of muscle.

9. Many agencies affect excitation and inhibition similarly.

10. The properties of *Mytilus* muscle and the motor end plate of striated muscle are in many respects similar.

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ERRATUM

Vol. XXII, *No.* 3, *September* 1945, *Sec. B*

Page 126, para 4, lines 5-6—"As this secondary contraction" *should read* " As this secondary decline of contraction....".

PHOTOPERIODISM AND SEX IN *ZEA MAYS*

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Introduction

The importance of day-length as an ecological factor has been greatly stressed for sometime past. Its seasonal and latitudinal variations have been found to affect to a remarkable degree, the growth, the morphological and the physiological developments in plants and in general the production of crops accounting to a great extent for their success and failure when all other conditions of environment are non-limiting. Sex, like many other developmental aspects of plant life, has also been reported to be greatly modified by different photoperiods or day-lengths and has thus been made the subject of great experimental study in recent years. In the present contribution an attempt has been made to study this important aspect of plant life by subjecting *Zea mays* plants to different photoperiodic treatments under low (normal) and high (additional) nitrogen conditions.

Experimental Procedure

The plants were grown from well sorted plump and sterilized seeds of Var. Jaunpur Yellow in 10" × 15" earthenwares filled with well mixed field soil. They were arranged into 4 different lots for differential illumination. The photoperiods used were of the order of 6, 12, 18 and 24 hours and were obtained by either additions to or subtractions from the normal daily duration. Artificial light was used to supplement daylight when necessary. The normal daily sunshine was, however, not a constant factor during experimentation. It varied from 13½ to 11¼ hours, yielding an average duration of 12 hours 11 minutes. Nitrogen in high nitrogen series was applied as ammonium sulphate at 10 gm. per pot in addition to that already present in the pot soil. Each of the above lots was thus redivided into two series representing low (normal) and high (additional) nitrogen respectively.

Effect of Different Photoperiods on Sex Reversal in Plants Grown under Low Nitrogen (Normal) Conditions

Photoperiodic changes were associated with sex reversal in *Zea mays* and the observations made in this regard are presented in Table I. The 12 hours' duration (normal) exposed plants were characterised with normal staminate and carpellate inflorescences. An exposure of plants to 6 hours a day caused a decided shift towards femaleness, such that in extreme cases the tassels practically were transformed into well-defined ears. This change was associated with the suppression of the branching system in the tassels, which consisted either of a single unbranched central or only slightly branched spike. This was the usual condition, though not a rule accompanying femaleness in reversed tassels (Fig. 1).

Further examination of 6 hours' exposed plants showed that about 60% of these had undergone sex reversal and although there was no close similarity in the degree of change affected, some silks with normal ovularies were present on almost every such tassel. On an average, 56 silks were produced per tassel but on account of inadequate fertilization only a few formed grains. The appearance of silks on these tassels was but at random presenting a bizarre appearance in comparison to tassels on common plants having normal phenotypic expression. The change was mainly effected in the basal part of the tassel only.

Table I

Showing degree of sex reversal in Zea mays *plants, subjected to different photoperiodic treatments under low (normal) and high (additional) nitrogen conditions*

Sex Reversion	Low (normal) Nitrogen Photoperiods				High (additional) Nitrogen Photoperiods			
	6 hrs.	12 hrs.	18 hrs.	24 hrs.	6 hrs.	12 hrs.	18 hrs.	24 hrs.
A. In tassel—								
% of sex reversed plants ..	60	..	18	19	71	..	22	25
Av. No. of silks per reversed tassel ..	56	..	31	43	78	..	56	58
B. In ear—								
% of sex reversed plants ..	..	..	21	28	..	..	11	16
Av. No. of staminate spikelets per reversed ear ..	..	..	120	136	..	..	85	71

It may be noted with interest that changes in sex expression were not abrupt and in fact all sorts of developmental stages between the staminate and the carpellate flowers were observed. One of the most conspicuous

changes recorded herein, was the suppression of pedicel of the pedicellate spikelets. Normally the staminate spikelets exist in pairs of which one is essentially a pedicellate one. During transformation from maleness to femaleness, majority of the tassels had transformed their pedicellate spikelets to the sessile form, inasmuch as cases were not uncommon wherein all spikelets were sessile. This change brought about a simultaneous change in the masculine nature of the tassel to the feminine of the ear. In certain cases, however, where change from stalked to sessileness did not take place, it were invariably the normal sessile spikelets which changed their character and formed grains. Where the two spikelets of the pair, on the other hand, happened to be sessile, both adopted a phenotypic female expression.

Reduction in the duration of light exposure to 6 hours was also associated with inadequate development of ears. In majority of cases such a condition obstructed fertilization so that the cobs on these plants were short and almost empty. These cobs developed long flexuous shanks consisting of a large number of internodes. A peculiar character of such shanks was the production of aerial roots at their nodes often clasping the shank to the stem. Production of large number of nodes and consequently of shucks was considered as indicative of a greater vegetative vigour transmuted to the shank by this short photoperiod (Fig. 2).

Reference to literature shows that the study of sex reversal has mostly been made by workers in relation to short light periods only while no reports seem available on the effect of supra-photoperiods. Long photoperiods in this investigation were also found to cause marked changes in sex expression in *Zea mays*. Of interest, it may be noted that unlike 6 hours' photoperiod, sex reversal under long light conditions occurred in both the directions, *viz.*, from maleness to femaleness (in tassels) and *vice versa* (in ears). It is, however, noticed that the change from maleness to femaleness under the influence of long light duration was not as large as under the short indicating thereby that the latter condition was more congenial to female expression in *Zea mays* (Table I and Fig. 1). It was formerly noted that a change into femaleness was associated with the reduction in the number of tassel branches and the same was found true when such transformations occurred under long light conditions. Working on *Crotalaria juncea*, Singh and Choudhri (1938) also observed a suppression in its branching system on deviations either way from the normal photoperiod. Whether the branching in tassels is a consequence of light supply or has something to do with the sexual expression in plants or is correlated with both cannot confidently be stated at the present stage of this enquiry.

Having described the susceptibility of tassels to photoperiodic changes in *Zea mays*, it is now aimed to describe the effect of such conditions on sex expression in ears. It has been seen that ears under short photoperiods were under-developed but at the same time were unaffected sexually. On the other hand, a change from femaleness to maleness took place as the period of light supply increased over 12 hours (Table I). The percentage of sex reversal under these conditions averaged 11 and 16 in 18 and 24 hours' photoperiods respectively.

The staminate spikelets on the modified ears were mostly concentrated in the apical region which had developed to form an abnormally long axis (Fig. 3). In the large majority of cases these spikelets were sessile and were arranged in double longitudinal rows as in the ear itself. This stage may be described as intermediate in the transformation of the female characters to maleness. Obviously, thus long light conditions seemed to favour maleness, while short light duration femaleness.

There was also a fair percentage of sexual change from maleness to femaleness even under long light periods which apparently seemed to favour the reverse phenomenon, *viz.*, the transformation of ears to tassels. This may probably be due to the unstable nature of maleness as compared to femaleness.

Effect of Different Photoperiods on Sex Reversal in Plants Grown under High (Additional) Nitrogen Conditions

In the previous experiment, the effect of light factor on plants grown under low (normal) nitrogen conditions has been to exert a great influence on sex expression. In this experiment the effect of nitrogen application in relation to its interactions with the different day-lengths has been tried. An increase in the number of pistillate spikelets on almost all modified tassels consequent to deficient light supply was the general feature observed in these additionally manured plants (Table I). This of course took place as a result of an accelerated reversion of maleness into femaleness under 6-hour illuminated series. Similarly there was somewhat increased reversion of staminate spikelets to pistillate ones even under long light photoperiods of 18 and 24 hours per diem.

Observations on pistillate inflorescence, on the other hand, showed no sex reversal in short photoperiod while under the influence of long photoperiods there was a significant reduction in the reversal percentage as compared to what was obtained in the low nitrogen (normal) series of plants.

Nitrogen application in *Zea mays*, thus evidently seemed to favour femaleness at the expense of maleness.

Additional nitrogen application had no effect on the sexual behaviour of plants grown under 12 hours' photoperiod, except that they were stouter as compared to plants under low nitrogen series. Nitrogen photoperiod interactions were, thus more, conspicuously depicted when day-lengths significantly deviated from the 12-hour duration.

Discussion

Observations in this investigation have shown that changes in day-length and nitrogen nutrition exert a great modifying effect on sexual expression in *Zea mays*. Sex modifications have also been reported in several crops by other workers (Edmond, 1930; Loehwing, 1933; Schaffner, 1935; Tiedjens, 1928). A lot of experimental data now exists on the effect of different environmental factors but hardly any complete information is available to account for the known complexities that occur in sexual expression. One thing, however, may be taken for certain, that the environmental factor brings about a change in what may be called the 'internal environment' of the plants. What is the nature of this change, is yet a mystery. Sach (1864, 1886) and Goldschmidt (1923) have attributed it to hormone and enzyme actions respectively, Kraus and Kraybill (1918) to disturbed carbon-nitrogen balance and Sabinin (1937) to pH of the sap in the tissues. Whatever may be nature of the change involved, it goes, however, without saying that the environment has got a great significance in the expression of sex in plants.

Summary

1. The investigation deals with the effect of different photoperiodic treatments (6, 12, 18 and 24 hours daily), on sex expression in *Zea mays* plants grown both under low N (normal) and high nitrogen (additional) conditions.

2. Twelve-hours' photoperiod plants were characterised with normal staminate and carpellate inflorescences. Under 6-hour photoperiod a conspicuous change was effected in tassels; many spikelets took up pistillate forms leading to grain formation. In long photoperiods contrariwise, reversal took place in ears where good many male spikelets developed in place of the carpellate ones.

3. A small change from staminate character to pistillate one also took place under long photoperiods. This is explained on the greater stability of the female characters in *Zea mays* than those of males.

4. Sex modifications were also initiated by heavy nitrogen nutrition to plants. This condition accelerated the change from maleness to femaleness in tassels but caused a relative inhibition in the sex reversal in the ear.

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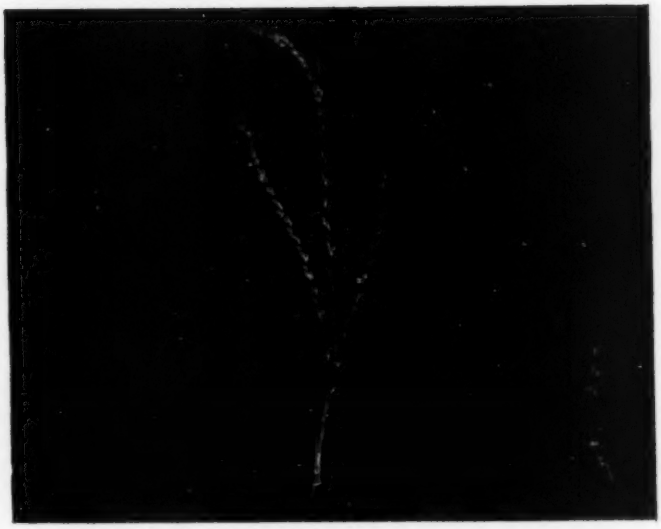

FIG. 1. Sex reversal in tassels from plants grown as pot culture under different photoperiods.

1. 6 hrs.—Unbranched tassel with sex reversal,
2. 12 hrs.—Branched tassel (normal), and
3. 24 hrs.—Slightly branched tassel with sex reversal.

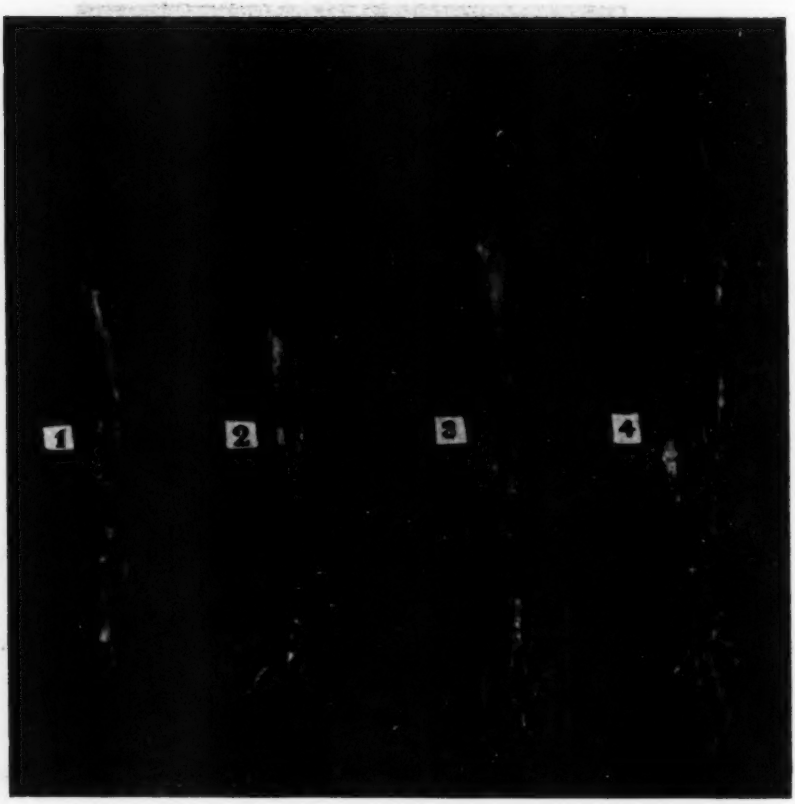

FIG. 2. Various gradational forms in ears from 6 hours illuminated plants photographed to show large shanks and characteristic root development upon shank nodes.

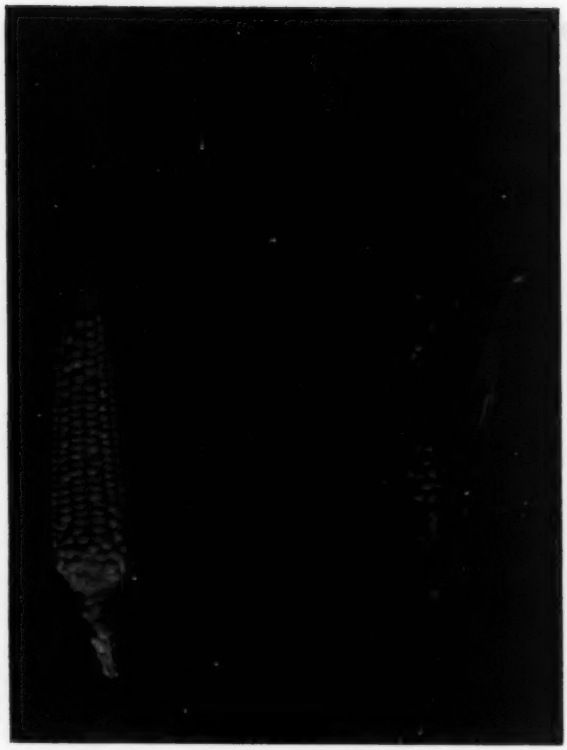

FIG. 3. *Zea mays* ears from plants grown under (i) 12 hours' illumination per day; (ii) continuous illumination (24 hours). Whereas the former of the two is a normal ear, the latter exhibits sex reversal particularly in the upper region resulting in an abnormal elongation of the cob.

CLINICAL OCCURRENCE OF PHLYCTENULAR KERATO-CONJUNCTIVITIS

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Introduction

Since the times of late Greek classics when a peculiar reaction of the conjunctiva and cornea, nodular in variety, appears to have been first recognised, a large volume of work has appeared concerning almost every aspect of phlyctenular kerato-conjunctivities. Detailed reports have appeared in recent times, showing that the disease was becoming uncommon, as in Great Britain (Walker, 1928; Ballantyne, 1936; Goulden, 1936), Australia (Barrett, 1938), and the United States of America (Goldenberg, 1930); while in France a continuous increase in the number of cases has been noticed. In most of the countries, however, the incidence of the disease increased during the World War 1914–18 (Sorsby, 1942, *a*).

Phlyctenular kerato-conjunctivitis is not uncommon in the Punjab. In the Eye Out-Patients' Department of the Mayo Hospital, Lahore, out of 54,818 cases applying for various eye diseases 484 (0·88%) were those of phlyctenulosis. In these cases an investigation into the clinical occurrence of this disease was undertaken, as similar records were not available for the Punjab. In the first part of this report our observations on the natural history of the disease are given, and in the second, examination of cases is discussed as far as it concerns the clinical occurrence of the disease.

Natural History

1. *Sex distribution.*—Out of 484 cases, males comprised 250 (51·6%) and females 234 (48·3%). This finding is at variance with the accepted view that the disease is more common in girls than in boys. In Table I are shown the number of cases, of either sex, compared with the latest published results, which record the incidence of disease in girls and boys as 60% and 39·1% respectively (Sorsby, 1942 *a*). It may be pointed out that Puscariu and Lazarescu (1935) after examining 684 cases gave the percentage of boys

TABLE I

Author	Total No.	Boys	Girls	Percentage distribution	
				Boys	Girls
Sorsby (1942) ..	1,392	544	848	39·1	60·9
Puscariu and Lazarescu (1935)	684	322	362	47·1	52·9
Present authors (1945) ..	484	250	234	51·6	48·3

and girls suffering from the disease as 47·1 and 52·9 (Table I), figures, which do not bring out the high incidence of disease in girls (as emphasised by other authorities), and thus tend to confirm our findings.

It was suggested by Guttman (1898) that the sex incidence of the disease was equal under five years of age, and it was after that age that the number of girls suffering from the disease increased gradually. Table II which

TABLE II

Sex	Percentage of total number in various age groups				
	0–4	5–9	10–14	15–19	Over 20
Boys (250) ..	10·1	25·7	19·3	8·5	35·9
Girls (234) ..	1·7	26·3	33·3	18·4	20·1

shows the percentage of cases in various age groups in boys and girls separately, brings out the fact that, in our series, number of cases in boys and girls was not equal under the age of five years. The incidence increases steadily, in either sex, up to 14 years after which there is a decline.

2. *Age distribution.*—It is generally held that phlyctenular ophthalmia is a disease of childhood and early adolescence. Cases reported in adults are usually considered as those of pseudo-phlyctens. In Table III (modified

TABLE III

Author	Percentage of total number in various age groups				
	0–4	5–9	10–14	15–19	Over 20
Guttmann (1898) ..	33·0	27·4	16·8	10·2	12·4
Krasso (1925) ..	8·2	15·4	17·5	20·2	31·1
Gronholm (1926) ..	30·0	21·0	21·0	16·0	10·0
Weekers (1929) ..	23·5	25·0	19·8	15·8	15·0
Rolet (1931) ..	22·5	21·5	26·0	11·0	18·9
Fasen-Moller (1936) ..	24·7	23·4	19·5	13·5	18·5
Forminopoulos (1940) ..	9·7	16·3	19·0	18·0	36·1
Present authors (1945) ..	6·1	26·0	26·0	13·2	28·5

from Sorsby) are shown the age groups in some of the large series published, and compared with those of our series. It is clear that by far the greater number of our cases occurred in the first two decades. There appears to be no unanimity among authors regarding any one age group in which the infection may be said to be commonest. In the series under report more than half the cases (52·0%) were seen in the age group 5–14 years, and cases under 5 years were distinctly uncommon (6·1%).

3. *Community distribution.*—It would be interesting to know the extent to which the members of various communities suffer from this disease in a heterogeneous population like that of the Punjab. The number of cases in each community are given in Table IV. From the perusal of this table it

TABLE IV

Community	Boys	Girls	Total	Percentage		
				Boys	Girls	Total
Muslims ..	148	136	284	52·1	47·8	58·6
Hindu ..	86	91	177	48·5	51·4	36·5
Sikhs ..	14	3	17	82·3	17·6	3·5
Others ..	2	4	6	33·3	66·6	1·2

appears that the children of the two major communities suffer from the disease roughly in proportion to their numbers in the population.

4. *Seasonal distribution.*—The consensus of opinion seems to favour spring as the time when maximum number of cases of phlyctenulosis are seen. All reports agree that the number of such cases is minimum towards the end of a calendar year (Sorsby, 1942 *a*). Table V gives the seasonal

TABLE V

Months	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.
Number ..	43	40	40	45	69	53	23	17	24	21	28	11
Percentage ..	10·3	9·6	9·6	10·8	16·6	12·8	5·5	4·1	5·7	5·0	6·7	2·6

distribution in 414 (out of 484) cases presented in this series, and indicates that the maximum number of cases occurred in the month of May. It may be pointed out that 70·0% of cases occurred in the first half of a year, and only about 30·0% in the latter half. This also confirms the view that the incidence of the disease declines appreciably towards the end of the year.

Examination of Cases

1. *Position of phlyctens.*—In 484 cases examined, 124 (25·6%) had simultaneous involvement of both eyes; in 190 (39·2%) right, and in 170 (35·1%) left eye was affected, 28 eyes (5·7%) had phlyctens all round the limbus, of which both eyes were involved in 10 cases, and either eye in 8 cases (4 right and 4 left).

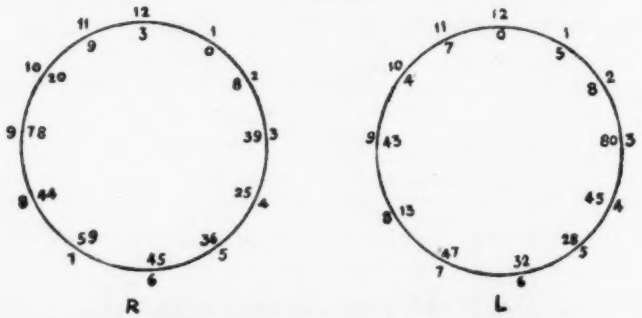
It has been stated that the phlyctens are most commonly seen on the outer quadrant of the limbus. The reason given by Hermann (1905) is as follows:—

"The seat of election of phlyctenules is in that section developed from the maxillary process and supplied by the second division of the fifth nerve (trigeminal) which borders on the joint territory at the outer quadrant. The second division of the fifth supplies the teeth of the upper jaw. There is surely enough irritation in the condition of teeth of poor children about the ages of 3 to 8, the years of greatest incidence, to disturb the trophic influences of other branches of the same division".

This view about the nerve supply of the limbus, would appear to be untenable in the light of our present day knowledge. It is believed that the anterior ciliaries (from the ophthalmic division of the trigeminal nerve) supply the circumcorneal zone of the conjunctiva (Wolff, 1940).

Our findings given in Table VI partially confirm Hermann's view about the seat of election of phlyctens in the majority of cases, inasmuch as the

Table VI



The number of cases, in each eye, are shown inside the circles

maximum number of phlyctens in the present series was seen at identical places in either eye, *viz.*, at 9 o'clock in the right eye and at 3 o'clock in the left. The various positions of the phlyctens in every case were recorded,

separately for each eye, on a series of charts depicting the face of a clock (Table VI). It will be seen from table that a total of 678 phlyctens were recorded in 484 cases; 366 in the right eye, and 312 in the left eye. In each the maximum involvement was seen in the lower half of the limbus between 3 o'clock and 9 o'clock; 326 plhyctens (89·0%) in the right and 288 (92·3%) in the left. 40 phlyctens (10·9%) in the right and 24 (7·6%) in the left eye were above this level.

2. *Incidence of keratitis.*—Less than one third of the cases showed any gross involvement of cornea, as seen with 2 per cent. fluorescine solution. Keratitis was present in 138 cases (28·0%) and in the rest 346 cases (71·5%) only conjunctiva was affected.

3. *Superadded infection.*—Mucopurulent conjunctivitis as a concomitant of phlyctenular kerato-conjunctivitis was surprisingly rare in our cases (as a clinical observation), being present only in 7·8%. We are thus unable to confirm the view held by some authorities (Duke-Elder, 1938; Parsons, 1942) that superadded infection was very common in phlyctenulosis.

4. *Cervical lymphadenitis.*—Enlarged glands in the neck may be due either to tuberculous infection or to nasopharyngeal sepsis; and both these are mentioned in the list of etiological factors of phlyctenulosis. Some degree of cervical lymphadenitis, on one or both sides, was present in 224 cases (46·2%), of which, in 136 (60·7%) the enlargement of glands was associated with nasopharyngeal sepsis, and in the rest 88 cases (39·2%) no cause of adenitis could be found in the nasopharynx. Table VII, depicting

TABLE VII

Degree of enlargement	Associated with sepsis		Un-associated with sepsis		Total	
	No.	Percent	No.	Percent.	No.	Percent.
+ ..	60	46·8	68	53·1	128	57·1
++ ..	22	30·5	50	69·4	72	32·1
+++ ..	6	25·0	18	75·0	24	10·7

these observations, shows that out of 224 cases 128 (57·1%) had slightly enlarged glands, 72 (32·1%) moderately so, and in 24 (10·7%) the glands were greatly enlarged. In those with slightly enlarged glands, 68 (53·1%) had nasopharyngeal sepsis, of the moderate adenitis group 50 (69·4%) and of the greatly enlarged glands groups 18 (75·0%) had some focus of infection in their nasopharynges. It is clear, therefore, that in more than half the cases of enlarged glands in phlyctenulosis some focus of infection in the

nasopharynx is detectable, and that in the rest the enlargement of glands is probably the result of tubercular infection.

5. *Nasopharyngeal sepsis.*—Nasopharynx was examined carefully, as a routine, in 294 cases. Out of these 171 (58·5%) had definite evidence of sepsis in the form of enlarged and sepsic tonsils, etc.

6. *Reaction to tuberculin.*—Mantoux test, 1 in 10,000 was done, as a routine, in 220 cases. As the work was carried out in the Out-Patient Department it was not possible to use stronger concentrations in those who did not react to the initial dose of 1 : 10,000 Wellcome's old tuberculin (human). In positive cases X-ray examination of the chest could not be done due to war-time restrictions. The results of Mantoux test are given in Table VIII.

TABLE VIII

Tipe of reaction	No.	Percentage
Negative ..	69	31·3
+ ..	43	19·5
++ ..	32	14·5
+++ ..	71	32·2
++++ ..	5	2·2

It will be seen from this table that positive tuberculin reaction was noticed in 68·7% of cases. It is quite likely that of the 31·3% negative cases a few more would have turned out as positive if they had been given stronger concentration of tuberculin. Even as they are, the figures show that over two-third of all cases were positive to tuberculin, a finding which is in entire agreement with that of others (Sorsby, 1942 *b*). In the population the number of tuberculin positive cases has been assessed as 25% (Taylor, 1944).

7. *Blood examination* and erythrocytic sedimentation rate were carried out in some cases but the results were inconclusive.

CONCLUSIONS AND SUMMARY

A report is presented of an investigation into the clinical occurrence of phlyctenular kerato-conjunctivitis in 484 cases. Although it was limited in scope, as it was carried out in an Out-Patients Department, and exhaustive examinations, clinical laboratory and rontgenographic could not be done, the data collected is considered enough to draw the following conclusions:—

1. Phlyctenular ophthalmia is not uncommon in the Punjab.
2. Girls are affected in equal numbers as compared with boys.
3. Maximum number of cases are seen in the age group 5 to 14 years.

4. Children of the major communities suffer from the disease roughly in proportion to their numbers in the population.

5. Maximum number of cases occur in the month of May, and 70 per cent. of cases occur in the first half of a calendar year.

6. The seat of election of phlyctens is the outer part of the limbus in either eye, at 9 o'clock in the right, and at 3 o'clock in the left. About 90 per cent. of phlyctens in either eye appear in the lower half of the limbus.

7. Keratitis is associated in little less than one-third of all cases.

8. Superadded infection is surprisingly rare.

9. In little less than half the cases the cervical lymph glands are enlarged. In half of such cases some focus of infection is detectable in the nasopharynx, and in the rest the cause of adenitis is probably tubercular infection.

10. Over half the cases have nasopharyngeal sepsis.

11. Two-thirds of all cases are sensitive to tuberculin. Desensitisation would, therefore, be a rational method of treatment in the majority of cases.

Acknowledgements

Thanks are due to Khan Bahadur Professor M. Bashir for his keen personal interest, and for kindly going through the rough draft of this paper. We are indebted, in addition, to several of our colleagues, in other departments, for help during this investigation, particularly to Dr. A. Rashid, D.L.O., and Dr. G. M. Taneja, P.C.M.S., of the Ear, Nose and Throat Out-Patients Department; Dr. Kartar Singh, P.C.M.S., Clinical Pathologist, Mayo Hospital, Lahore; and Khan Sahib Dr. Riaz Ali Shah, D.P.H., and his assistants in the Rai Bahadur Amar Nath Tuberculosis Institute, Lahore.

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AMIDE SYNTHESIS IN PLANTS

II. Amino-acid Changes in Germinating Seedlings

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Our knowledge of amino-acid changes during protein regeneration in seedlings is based almost entirely on the researches of Schulze. In the absence of adequate analytical methods Schulze had to rely upon isolation of individual amino-acids for their estimation—a procedure which even in his masterly hands was only of limited application and incapable of yielding quantitative results. The present work was carried out with the object of utilizing the better methods now available to ascertain more precisely the changes occurring in the proteinogenous constituents of seedlings. Three species of leguminosæ, namely, *Dolichos biflorus*, *Phaseolus mungo* and *Cicer arietinum*, were used for experiments and the changes in the following constituents during germination and seedling growth were studied:

1. Extractable and non-extractable N.
2. Protein, non-protein, aspearagine, glutamine, ammonia, amino, peptide and amide N in the extractable material.
3. Total amide, dicarboxylic acids, bases, arginine, histidine, cystine, lysine, mono-amino mono-carboxylic acids, and mono-amino acids, after complete hydrolysis.

Experimental

The seeds were soaked for 24 hours in water and germinated in flats on sterile sand in a room with plenty of diffused light and watered with tap water. Analyses were carried out every three days till food reserves in the cotyledons were exhausted and the seedlings showed signs of decay (usually about three weeks). In each series the same number of seedlings, between 200 and 400, was removed for the analysis. The particles of sand were gently washed off under the tap and the adherent moisture removed by means of filter-paper. A small portion of the seedlings was taken for dry weight and total nitrogen determined. The rest was used for water extraction. It was found that the most satisfactory method of extraction was to grind

the seedlings using a granite mortar and pestle as this obviated the necessity of adding sand. The mortar was previously cooled in the refrigerator and the seedlings quickly ground up with ice-cold water. A uniform suspension was thus obtained which was centrifuged for 15 minutes in a cooled centrifuge. After pouring off the supernatent liquid the residue was washed three times with water and the combined extracts and washings made up to volume. The extract (E) and residue (R) were separately analysed as follows:

An aliquot of the extract was deproteinised by rapidly raising the temperature to 80° C. and cooling in ice. The protein precipitated was separated by filtration and washed with cold water. The filtrate and washings were made up to volume and used for estimation of N, amino N, free ammonia

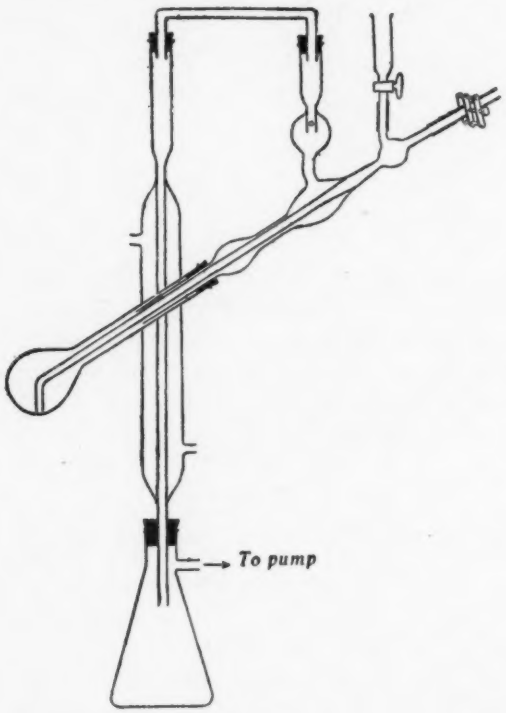

FIG. 1

(Parnas and Klisiecki, 1926), asparagine and glutamine (Chibnall and Westall, 1932; Vickery, *et al.*, 1935). For determination of amide and peptide N an aliquot was hydrolysed under the usual conditions, *i.e.*, for 18 hours after

addition of sufficient concentrated hydrochloric acid to give 20% concentration. The hydrolysate was repeatedly evaporated to dryness *in vacuo* after addition of water to remove most of the acid. For estimation of amino N in the hydrolysate it was made up to volume, an aliquot made alkaline to phenolphthalein and evaporated nearly to dryness *in vacuo* to expel ammonia. The residue was taken up in water containing a little acetic acid and used for determination of amino N. In dealing with the small quantities of material involved in these experiments, it was found convenient to use a modified form of the micro-Kjeldahl's distillation apparatus for removal of acid and ammonia. The apparatus is illustrated in Fig. 1 which explains itself. Using this apparatus the hydrolysis as well as the distillations to remove acid and ammonia could all be carried out in the same Kjeldahl flask which was either 50 ml. or 100 ml. in capacity according to the quantity of material dealt with.

The whole of the unextractable residual material (R) and the main bulk of the extract (E) after the removal of aliquots as mentioned above, were separately analysed after complete hydrolysis with 20% hydrochloric acid for 18 hours. E was acidified with a little hydrochloric acid and evaporated to a convenient volume before hydrolysis. The nitrogen partition was carried out in the usual way with the modifications generally used in this Laboratory as described by Krishnan and Krishnaswamy (1939). Arginine and histidine were determined in the phosphotungstic acid precipitate colorimetrically, arginine by the method of Klein and Taubock (1932) and histidine according to Hanke and Koessler (1920). Cystine was determined in a separate portion of the hydrolysate by the Folin Marenzi method as modified by Tompsett (1931).

Results

Table I

Distribution of N in Dolichos biflorus

(Values as % of total N)

Age of seedlings in days	1	4	7	10	13	16	19	22
Total N in 400 seedlings (mg.)	378·7	339·9	347·7	348·0	373·5	348·4	333·7	349·6
Extractable N % ..	93·2	80·4	67·5	70·6	75·9	78·8	72·6	79·3
Protein N in extract %	69·6	32·9	13·7	11·1	9·5	9·8	4·8	8·3
Non protein N in extract %	23·6	47·5	53·8	58·5	65·4	69·0	67·8	71·0
Residual N % ..	6·8	19·6	32·5	29·4	24·1	21·2	27·4	20·7

TABLE II

Distribution of N in Phaseolus mungo

(Values as % of total N)

Age of seedlings in days	1	4	7	10	13	16
Total N 300 seedlings (mg.) ..	534·5	486·2	511·2	517·2	499·6	505·5
Extractable N % ..	94·2	76·6	77·6	82·9	85·2	84·9
Protein N in extract % ..	82·8	34·7	19·9	8·6	12·7	6·7
Non-protein N in extract % ..	11·4	41·9	57·6	74·3	72·5	78·2
Residual N % ..	5·8	23·4	22·4	17·1	14·8	15·1

TABLE III

Distribution of N in Cicer arietinum

(Values as % of total N)

Age of seedlings in days	1	4	7	10	13	16
Total N in 200 seedlings (mg.) ..	699·4	710·4	707·3	634·3	642·7	594·0
Extractable N % ..	86·5	75·5	74·3	74·8	76·5	81·2
Protein N in extract % ..	68·4	38·1	19·9	8·8	7·0	8·1
Non-protein N in extract % ..	18·1	37·4	54·4	65·9	69·5	73·1
Residual N % ..	13·5	24·5	25·7	25·2	23·5	18·8

TABLE IV

Analysis of non-protein N fraction of Dolichos biflorus

(Values expressed as % of total non-protein N and, in brackets, as % of total N of seedlings)

Age of seedlins in days	1	4	7	10	13	16	19	22
Total non-protein N in aqueous extract mg.	89·6	161·5	187·1	203·6	244·2	240·4	226·9	248·2
Ammonia N % ..	0·3 (0·1)	2·6 (1·2)	2·3 (1·2)	1·1 (0·6)	1·9 (1·3)	2·2 (1·5)	1·8 (1·2)	1·5 (1·1)
Aspariagine amide N %	7·1 (1·7)	14·0 (6·6)	19·8 (10·7)	21·0 (12·5)	20·2 (13·4)	17·6 (12·1)	15·5 (10·5)	16·6 (11·8)
Glutamine amide N %	1·1 (0·3)	1·9 (0·9)	3·4 (1·8)	3·8 (2·3)	3·9 (2·6)	5·2 (3·6)	4·9 (3·4)	5·4 (3·9)
Amino N % ..	36·9 (8·7)	49·9 (23·7)	49·9 (26·9)	43·2 (25·3)	34·6 (22·6)	34·6 (23·5)	30·1 (20·8)	25·0 (17·7)
Peptide N % ..	25·8 (6·1)	11·0 (5·1)	4·3 (2·3)	..	..	..	..	..
Amide N % ..	11·9 (2·8)	31·3 (14·8)	39·7 (21·3)	45·4 (26·5)	46·7 (30·5)	46·4 (31·4)	41·6 (28·6)	45·6 (32·4)

TABLE V

Analysis of non-protein N fraction of Phaseolus mungo

(Values expressed as % of total non-protein N, and in brackets, as % of total N of seedlings)

Age of seedlings in days	1	4	7	10	13	16
Total non-protein N in aqueous extract (mg.)	60·8	203·6	294·4	383·9	362·2	395·5
Ammonia N %	2·4 (0·3)	2·1 (0·9)	2·0 (1·2)	1·1 (0·8)	1·6 (1·1)	3·2 (2·5)
Asparagine amide N %	6·1 (0·7)	22·3 (9·3)	19·7 (11·3)	17·1 (12·7)	17·3 (12·5)	15·0 (11·7)
Glutamine amide N %	2·4 (0·3)	2·5 (1·1)	2·5 (1·4)	3·1 (2·3)	3·9 (2·9)	3·2 (2·5)
Amino N %	28·2 (3·2)	39·2 (15·4)	41·1 (23·6)	32·00 (23·7)	31·1 (22·5)	26·6 (20·8)
Peptide N %	25·2 (2·9)	.. ..	.. ..	2·4 (1·8)	1·5 (1·1)	1·9 (1·5)
Amide N %	16·3 (1·9)	.. ..	.. ..	38·1 (28·3)	46·5 (33·7)	33·8 (26·4)

TABLE VI

Analysis of non-protein N fraction of Cicer arietinum

(Values expressed as % of total non-protein N and, in brackets, as % of total N of seedlings)

Age of seedlings in days	1	4	7	10	13	16
Total non-protein N in aqueous extract (mg.)	126·6	265·6	384·4	418·0	446·5	434·1
Ammonia N %	0·6 (0·1)	1·6 (0·6)	1·3 (0·7)	1·1 (0·7)	1·1 (0·8)	1·2 (0·9)
Asparagine amide N %	5·2 (0·9)	13·1 (4·9)	22·2 (12·1)	28·7 (19·0)	27·4 (19·0)	29·6 (21·7)
Glutamine amide N %	1·0 (0·2)	1·6 (0·6)	1·3 (0·7)	1·3 (0·9)	1·9 (1·3)	2·4 (1·8)
Amino N %	24·2 (4·4)	41·0 (15·3)	52·8 (28·7)	49·5 (32·4)	55·9 (38·9)	48·2 (35·3)
Peptide N %	21·7 (3·9)	4·6 (1·7)	.. ..	.. ..	.. ..	.. ..
Amide N %	13·9 (2·5)	22·4 (8·4)	31·1 (16·9)	40·6 (26·7)	39·1 (27·1)	44·7 (32·6)

TABLE VII

Analysis of whole seedlings of Dolichos biflorus

(Values expressed as % total N of seedlings)

Age of seedlings in days	1	4	7	10	13	16	19	22
Total N in 400 seedlings (mg.)	378·7	339·9	347·7	348·0	373·5	348·4	333·7	349·6
Dicarboxylic acids %—								
Extract ..	20·6	19·7	17·6	19·5	19·1	19·0	18·2	19·3
Residue ..	0·8	5·0	8·0	6·1	5·5	3·9	5·7	4·1
Total ..	21·4	24·7	25·6	25·6	24·6	22·9	23·9	23·4
Amide N %—								
Extract ..	8·9	16·7	20·9	25·0	28·1	31·4	28·6	30·7
Residue ..	0·9	2·3	4·1	4·2	3·6	3·5	4·1	3·0
Total ..	9·8	19·0	25·0	29·2	31·7	34·9	32·7	33·7
Bases %—								
Extract ..	26·3	19·9	11·3	8·5	7·2	8·5	9·0	8·9
Residue ..	1·4	4·6	8·7	8·0	6·2	5·2	6·5	5·1
Total ..	27·7	24·5	20·0	16·5	13·4	13·7	15·5	14·0
Arginine %—								
Extract ..	11·6	7·0	4·0	1·0	0·0	0·0	0·0	0·0
Residue ..	0·8	1·2	3·1	3·3	2·8	2·3	3·3	2·6
Total ..	12·4	8·2	7·1	4·3	2·8	2·3	3·3	2·6
Histidine %—								
Extract ..	2·4	1·6	1·3	1·3	1·2	1·2	1·2	1·0
Residue ..	..	0·3	0·8	0·3	0·2	0·4	0·6	0·4
Total ..	2·4	1·9	2·1	1·6	1·4	1·6	1·8	1·4
Cystine %—								
Extract ..	0·6	1·6	0·6	0·3	0·4	0·2	0·3	0·1
Residue ..	0·3	0·6	0·8	1·9	1·4	1·1	1·2	0·9
Total ..	0·9	2·2	1·4	2·2	1·8	1·3	1·5	1·0
Lysine%—								
Extract ..	11·7	9·8	5·4	5·8	5·6	7·0	7·5	7·8
Residue ..	0·3	2·6	4·1	2·6	1·8	1·3	1·5	1·2
Total ..	12·6	12·4	9·5	8·4	7·4	8·3	9·0	9·0
Monoamino carboxylic acids %—								
Extract ..	31·8	25·0	14·5	11·8	10·1	10·6	10·.	11·0
Residue ..	3·6	6·8	10·7	9·8	7·1	8·1	9·2	7·7
Total ..	35·4	31·8	25·2	21·6	17·2	18·7	19·4	18·7
Non-amino acids %—								
Extract ..	10·2	8·8	7·1	4·7	6·1	7·7	9·3	9·4
Residue ..	1·4	2·7	2·6	2·0	2·5	1·1	2·0	1·8
Total ..	11·6	11·5	9·7	6·7	8·6	8·8	11·3	11·2

TABLE VIII

Analysis of whole seedlings of Phaseolus mungo

(Values expressed as % total N of seedlings)

Age of seedlings in days	1	4	7	10	13	16
Total N in 300 seedlings (mg.)	534·5	486·2	511·2	517·2	499·6	505·5
Dicarboxylic acids %—						
Extract ..	22·5	23·1	25·7	23·8	24·1	24·6
Residue ..	0·6	4·9	6·7	4·6	4·1	4·7
Total ..	23·1	28·0	32·4	28·4	28·2	29·3
Amide N %—						
Extract ..	10·5	17·5	25·7	29·7	30·7	32·5
Residue ..	0·7	2·8	3·3	2·0	1·8	2·6
Total ..	11·2	20·3	29·0	31·7	32·5	35·1
Bases %—						
Extract ..	32·4	20·8	16·4	16·2	15·4	16·1
Residue ..	0·8	6·2	6·2	4·3	3·9	4·0
Total ..	33·2	27·0	22·6	20·5	19·3	20·1
Arginine %—						
Extract ..	13·7	9·1	8·4	9·2	8·7	9·1
Residue ..	0·2	2·9	2·9	2·4	1·9	1·9
Total ..	13·9	12·0	11·3	11·6	10·6	11··
Histidine %—						
Extract ..	3·9	3·1	2·9	2·9	2·7	2·5
Residue ..	0·0	0·5	0·5	0·4	0·5	0·5
Total ..	3·9	3·6	3·4	3·3	3·2	3·0
Cystine %—						
Extract ..	1·0	0·8	0·6	0·6	0·5	0·4
Residue ..	0·3	0·5	0·6	0·5	0·5	0·6
Total ..	1·3	1·3	1·2	1·1	1·0	1·0
Lysine %—						
Extract ..	13·8	7·8	4·3	3·5	3·4	4·0
Residue ..	0·3	2·3	2·1	1·1	1·1	1·1
Total ..	14·1	10·1	6·4	4·6	4·5	5·1
Monoamino carboxylic acids%—						
Extract ..	25·6	16·3	12·4	9·9	7·9	7·2
Residue ..	2·8	7·6	7·4	6·0	5·2	4·5
Total ..	28·4	23·9	19·8	15·9	13·1	11·7
Non-amino acids%—						
Extract ..	3·4	4·3	5·4	5·6	6·9	7·7
Residue ..	1·1	1·9	0·8	1·4	1·0	0·8
Total ..	4·5	6·2	6·2	7·0	7·9	8·5

TABLE IX

Analysis of whole seedlings of Cicer arietinum

(Values expreseed as % total N of seedlings)

Age of seedlings in days	1	4	7	10	13	16
Total N in 200 seedlings (mg.)	699·4	710·4	707·3	634·3	642·7	594·0
Dicarboxylic acid %—						
Extract ..	21·2	21·5	26·8	30·4	30·6	35·8
Residue ..	2·0	4·1	6·3	7·5	7·4	6·6
Total ..	23·2	25·6	33·1	37·9	38·0	42·4
Amide N %—						
Extract ..	11·9	12·4	18·2	28·6	28·9	32·3
Residue ..	1·6	2·9	3·4	5·0	5·0	4·6
Total ..	13·5	15·3	21·6	33·6	33·9	36·9
Bases %—						
Extract ..	28·9	20·9	13·1	10·8	10·0	8·7
Residue ..	3·0	5·6	6·3	5·5	4·8	3·3
Total ..	31·9	26·5	19·4	16·3	14·8	12·0
Arginine %—						
Extract ..	19·8	9·1	4·3	2·8	2·7	2·4
Residue ..	2·3	3·0	2·7	1·3	1·2	1·0
Total ..	22·1	12·1	7·0	4·1	3·9	3·4
Histidine %—						
Extract ..	2·3	1·8	1·5	1·9	1·1	1·3
Residue ..	0·1	0·2	0·4	0·5	0·3	0·4
Total ..	2·4	2·0	1·9	2·4	1·4	1·7
Cystine %—						
Extract ..	0·7	0·6	0·9	0·7	0·9	0·6
Residue ..	0·3	0·6	0·6	0·8	0·4	0·4
Total ..	1·0	1·2	1·5	1·5	1·3	1·0
Lysine %—						
Extract ..	6·2	9·4	6·4	5·3	5·3	4·4
Residue ..	0·3	1·8	2·7	3·0	2·8	1·6
Total ..	6·5	11·2	9·1	8·3	8·1	6·0
Monoamino carboxylic acids %—						
Extract ..	24·8	19·6	15·5	9·2	8·2	7·6
Residue ..	5·9	9·0	8·0	6·6	6·1	4·6
Total ..	30·7	28·6	23·5	15·8	14·3	12·2
Non-amino acids %—						
Extract ..	1·8	1·5	2·1	0·7	1·2	1·7
Residue ..	1·2	3·0	1·4	0·4	0·6	0·5
Total ..	3·0	4·5	3·5	1·1	1·8	2·2

Discussion

The results of the analyses are given in three separate tables for each seedling (Tables I-IX). In one table is given the distribution of N as between: (*a*) the water extractable portion and the insoluble residue and (*b*) the protein and non-protein fractions of the aqueous extract. In the next table is given the analysis of the various constituents of the protein-free aqueous extract. Analytical values are given for free ammonia, asparagine, glutamine and amino N as a per cent. (*a*) of the total N of the protein-free solution, and (*b*) of the total N of the whole seedling. The third table contains the results obtained after complete hydrolysis, the values obtained with the extract and the residue being added up to give the value for the whole seedling.

Interpreting the results, it is necessary to bear in mind that in the seedling processes growing in opposite directions are taking place, that there is breakdown of reserve protein of the seed and the new formation of the protoplasmic protein of the plant. Tables I, II and III show that there is an extremely rapid solubilization of reserve protein in the earliest stage of germination. Maximum values for soluble N are obtained within the first 24 hours and amount to about 90% of the total N. During seedling growth the quantity gradually decreases presumably due to resynthesis into insoluble plant protein. The soluble N, however, remains high—between 70% and 80%—throughout the experimental period. In the early stage of germination there is a high proportion of soluble protein which decreases rapidly indicating that degradative changes are pre-dormant during this period of seedling growth and that new synthesis of protein takes place only after complete hydrolysis of seed protein. This is clear from the values for the non-protein N in the aqueous extracts which steadily increase in the course of the experiment to between 70 to 80% in all the three species studied.

Tables IV, V and VI showing the results of the analysis of the protein-free aqueous extract gives some idea of the forms in which the soluble nitrogenous material is present. The amount of free ammonia present at any time is small and does not show much variation with time. In all three species the chief nitrogenous constituents are seen to be free amino-acids and amides which together reach values as high as 90% of the non-protein and about 60% of the total N of the seedlings. The increase in amides is due mainly but not entirely to asparagine and glutamine. Both these amides which to begin with are present only in small amounts increase during germination to many times their initial values but asparagine is by far the preponderant constituent. The accumulation of asparagine in these

experiments takes place under normal conditions of growth and not as a result of etiolation as in many of Schulze's experiments. The formation of asparagine must therefore be considered a normal metabolic process contrary to the suggestion made by certain authors (*cf.*, Prianischnikow, 189; Mothes, 1929) that it arises only under conditions when amino-acids cannot be utilized for protein synthesis due to lack of carbohydrate or other causes. The fact that there is an appreciable gap between the amide N values obtained by complete hydrolysis and the sum of the asparagine and glutamine amide N would indicate the presence of a third amide in addition to asparagine and glutamine. Evidence will be presented in a subsequent paper to show that there is in fact a third amide, namely, urea, present in these seedlings. In *Phaseolus mungo* and *Dolichos biflorus* asparagine and glutamine show a rapid increase followed by a gradual fall (Figs. 2 and 3). The

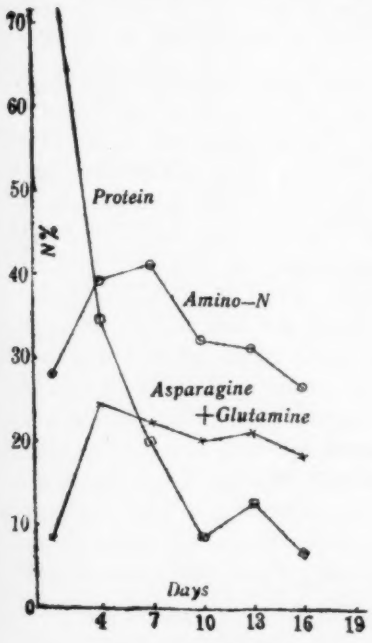

FIG. 2. *Dolichos biflorus*

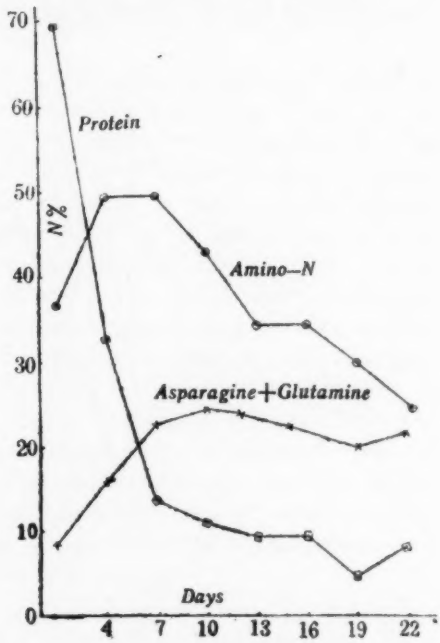

FIG. 3. *Phaseotus mungo*

value at the end of the experiment however remains quite high. The initial rapid rise in these amides is to be explained as a result of their formation from amino-acids and the subsequent fall to their utilization in protein synthesis. Amino N values also show an initial rapid increase, but also a subsequent

rapid fall, the final values reached being lower than the initial values. It is obvious that the amino N at any time represents the net result of two processes, the formation of amino-acids by degradation of protein, and the conversion of the former into amides. This is clear from the graphs in Figs. 2, 3 and 4 which depict a very rapid disappearance of protein in the seedling at a time during which there is rapid increase in the free amino groups, and of asparagine and glutamine. Amino N begins to fall at a stage when there is no further decrease in the quantity of soluble protein. The picture in regard to *Cicer arietinum* is slightly different from those of the other two seedlings. The initial breakdown of protein and the increase in asparagine, glutamine and in amino N. are observed here also but the subsequent stages marked by the rapid fall in amino-acids and the gradual decrease in amides is not evident. This is probably due to the fact that the seeds contain much larger reserves of food material and that the experiments had not been carried out for a sufficiently long period.

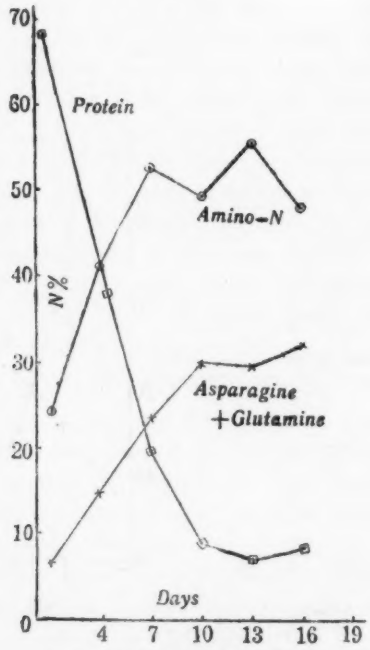

FIG. 4. *Cicer arietinum*

The peptide N values are unfortunately incomplete but such figures as were obtained would indicate the absence of appreciable quantities of inter-

mediate degradation products such as proteoses or peptones except at the earliest stages.

In discussing the results of the analyses of the seedlings after complete hydrolysis, it has to be remembered that the accuracy possible in the determination of amino-acids in hydrolysates of whole plants is bound to be of a lower order than that attainable with purified proteins. Attention has been drawn to this fact by Lugg (*cf.*, Chibnall, 1939) who has discussed some of the errors arising in protein analysis from the presence of large quantities of carbohydrate material. Our experience in the course of this work bears out the findings of Pollard and Chibnall (1934) and of Bailey (1937) with regard to the erratic results obtained in the estimation of cystine under these conditions. The histidine values also are not entirely satisfactory as it was not always found possible to obtain a proper colour comparison. There is no reason however to doubt the accuracy of the colorimetric estimation of arginine as applied to seedling hydrolysates.

Allowing for all experimental errors, certain conclusions can safely be drawn from the present experiments. A striking fact is that in none of the seedlings is there a parallelism between the changes in the dicarboxylic acids and amides (Tables VII, VIII and IX). In all three species in the early stages of germination, the dicarboxylic acids are much in excess of the amides. Only in *Cicer arietinum* do both dicarboxylic acids and amides show a parallel increase. In *Phaseolus mungo* and *Dolichos biflorus* there are no significant increases in the quantity of dicarboxylic acids, while the amides increase rapidly and are in the later stages considerably in excess. The reason for the excess of amide over the dicarboxylic acids is to be explained as has already been pointed out by the presence of urea. The constancy of the dicarboxylic acid content, as shown in Tables VII and VIII for *Phaseolus mungo* and *Dolichos biflorus*, considered in conjunction with the rapid increase of asparagine taking place at the same time, makes it highly probable that a conversion of glutamic acid into aspartic acid according to the scheme of reactions postulated in a previous paper (Damodaran and Venkatesan, 1941) takes place in these two seedlings.

Apart from the dicarboxylic acids all the amino acid fractions and individual amino acids show a very marked decline from their initial values in conformity with the hypothesis of the secondary formation of aspartic acid and glutamic acid from other amino acids. This decrease in amino acids takes place only in the extractable nitrogen of the seedlings. In the residual nitrogenous material all amino acid fractions show in general a slight increase due presumably to new synthesis of plant protein. The most marked fal

is shown by the basic and monoamino-monocarboxylic acid fractions, and among the individual amino acids by arginine. The non-amino acids either do not show much change (*Cicer arietinum*) or a small decrease (*Dolichos biflorus*) or even a small increase (*Phaseolus mungo*). The significance of this is however not clear. The values for arginine especially in *Dolichos biflorus* and *Cicer arietinum*, are very striking. In the former, after the tenth day there is no detectable amount of arginine in the extract and only a fraction of the initial arginine is present in the latter. There can be no doubt that this amino-acid plays an important part as a precursor of amides. Histidine which was not found in high concentration at any time and lysine, which initially had a high value, also showed a definite fall. Cystine showed no clear trends as was to be expected from the analytical difficulties already referred to.

Summary and Conclusions

A study has been made of the amino-acid changes taking place during protein regeneration in seedlings of *Dolichos biflorus*, *Phaseolus mungo* and *Cicer arietinum*.

The first metabolic change observed is the rapid solubilization and hydrolytic breakdown of the seed protein. The soluble nitrogen reaches a maximum within 24 hours of germination. This is followed by hydrolysis of protein and the conversion of the greater part of it into amino-acids and subsequently to amides. During seedling growth all amino-acid fractions decrease with the exception of the dicarboxylic acids and their amides. Both asparagine and glutamine increase to many times their initial values but the quantity of glutamine is in all cases much smaller than that of asparagine. From the conditions under which the plants were grown asparagine formation must be considered a normal stage in protein regeneration in plants.

The presence in the seedlings of a third amide in addition to asparagine and glutamine can be inferred from the following facts:

(1) The amide N in the aqueous extracts of the seedlings is greater than can be accounted for by the sum of asparagine and glutamine amide N present.

(2) Amides are present in excess of the dicarboxylic acids, especially in *Dolichos biflorus* and *Phaseolus mungo*.

In *Dolichos biflorus* and *Phaseolus mungo* asparagine accumulation takes place without a corresponding increase in dicarboxylic acids indicating the possibility of a conversion of glutamic acid into aspartic acid.

The strikingly rapid disappearance of arginine observed in some of the seedlings suggests that this amino-acid plays a special role as a precursor of amides.

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PROTEOLYTIC ENZYMES OF THERMOPHILIC BACTERIA—PART I

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Thermophilic bacteria are widely distributed in soil, etc., and are responsible for a large number of biological processes which are of importance in nature, in agriculture and in certain industries. Proteolytic breakdown is an essential feature of many of these processes. These bacteria can grow at high temperatures often close to the coagulation temperature of their albumins. In Beccari system of garbage fermentation, for example, temperatures above 50° C. are reached and thermophilic bacteria undoubtedly play a part liquefying the proteins in the initial stages and decomposing amino acids in the later stages. de Kruyff (1910) and Bergey (1919) have isolated from soil, manure, dust, etc., several species of thermophilic bacteria, many of which were of a proteolytic nature. Rabinowitsch (1895) has found thermophiles in soil and Weinzirl (1919) found *B. ærothermophilus* in canned string beans. Pricket (1928) has found *B. ærothermophilus* wide spread in hay, dust, beef extract, water, milk and agar. Thermogenic fermentation in the production of silage, processing of tobacco, cacoa and coffee as well as the harmful heating up of hay is well known and according to James *et al.* (1928) nitrogen metabolism and thermogenesis are interrelated. According to Dunez (1933) nitrogen in the form of albumin and its degradation products plays an important role in the fermentation of manure piles by thermophilic bacteria. Damon and Feirer (1925) have found aerobic and proteolytic thermophils in horse manure. Hops and oil-seeds often heat up on account of the activity of thermophilic bacteria. Microbial proteolytic thermogenesis of wool has been studied by Barker (1929). It is well known that thermophilic bacteria and molds are the active agents in manure fermentation. Damage caused to canned meat, fish, etc., by thermophilic bacteria is well known and Rokusho and Fukutome (1937) ascribed a type of spoilage of soya beans to the proteolytic activity of thermophilic bacteria.

The nature and properties of microbial proteinases and their mode of formation is still imperfectly understood. Considerable amount of work is still necessary with different types of micro-organisms and by using more reliable

and more decisive methods of analysis and experimental methods. Proteolytic enzymes of thermophilic bacteria do not seem to have been investigated at all. These bacteria thrive at unusually high temperatures and this fact, coupled with their practical importance, renders them an intriguing subject for study even apart from any theoretical interest in the nature of microbial proteinases in general. The present investigations are devoted to a study of the proteinases of three typical thermophilic bacteria:—

Bacillus thermophilus
Bacillus aerothermophilus
Bacillus thermoacidurans.

Cultures of these organisms were obtained from the Lister Institute, London.

Methods of Analysis

The methods of analysis employed by a majority of the previous workers in the study of microbial proteinases do not seem to have been quite satisfactory. For instance, a large number of investigators have estimated the amount of α-amino acids liberated by the proteinase without having previously verified the absence of poly- and dipeptidases in their enzyme extracts. Besides estimation of liberated amino acids alone does not throw any light on the initial stages of proteolytic breakdown when the protein undergoes profound physical changes without any appreciable increase in α-amino nitrogen. It would be more satisfactory to use a scheme of analysis whereby both initial and final stages of proteolytic action can be determined. The analytical schemes for various proteins actually adopted in the present study comprise firstly the determination of the initial stage of proteolysis, secondly the final stage, *i.e.*, when hydrolysis has ceased or has become very slow an in addition the determination at stages intermediate between the intitial and final stages.

The most convenient method for studying the initial stages must necessarily depend upon the physico-chemical and colloidal properties of the protein. In the present investigations a sensitive method for gelatin solutions was afforded by the measurement, in an Ostwald viscometer, of the progressive fall in viscosity of the solution in the presence of a proteinase. Viscosity/time curves were plotted and when extrapolated to zero time they gave the relative initial speed of hydrolysis which is a good measure of the very first stages of proteolysis of gelatin. Whenever parallel experiments were to be conducted at different hydrogen-ion concentrations then due corrections were made for the effect of pH on the specific viscosity of gelatin solutions. Corrections due to any effect of buffer, heating, etc., can be

ignored as these conditions are always reproducible. In addition to determining the initial speed of hydrolysis the per cent. fall in initial viscosity in a definite period of time (30 minutes) was also calculated from the same set of observations and this gave an idea of the progress of proteolysis.

With the albumins the initial stages of hydrolysis were followed by precipitating the unaltered protein by boiling the solution after bringing it to the isoelectric point of the protein. A small quantity of pure sodium chloride was added to the solution before boiling in order to increase the range of maximum coagulability of the unaltered albumin to some extent on both sides of the isoelectric point, thus avoiding the extra time and labour necessary to bring the solution exactly to the isoelectric point of the albumin. The intermediate stage of proteolytic hydrolysis was determined by precipitation with trichloracetic acid.

The final stage of proteolytic breakdown in case of all proteins was studied by estimating the increase of free α-amino acids by using the micro Van Slyke method, due corrections being made for the auto-hydrolysis of the proteinase solution.

Protfinase Solutions

The bacteria were cultivated in nutrient broth by incubation at 50° C. for thirty-six to forty-eight hours. The cultures were centrifuged and filtered through Chamberland candles yielding cell free filtrates which were proteolytically very active and were used directly.

Substrates

Previous workers have generally used only one kind of protein in their investigations on microbial proteinases. The results obtained by them therefore do not appear to be based on very firm foundations. 'Bacto' gelatin flakes, Merck's dried egg albumin and casein (Hammersten) were the three proteins employed in the present study. In addition some experiments were made with serum albumin and with denatured albumin prepared in the laboratory. Egg albumin solutions were dialysed in parchment bags in ice cold distilled water before mixing with the buffer solution in order to remove the lower degradation products which accompanied the true albumin. Previous investigators do not seem to have taken this precaution. McIvaine's citrate-phophate mixtures were used as buffer as they do not hinder proteolysis, compare Weil and Kocholaty (1937).

Experimental Methods

Several stoppered test-tubes containing 10, 20 or 30 ml. of the protein solution of the required concentration and pH were prepared with the buffer

and 1% phenol or a drop of toluene per tube. These were brought to 38–39° C. in an incubator before starting an experiment. A test-tube was taken out, 1 ml. or 2. ml. of the enzyme solution was run in and the test-tube was shaken. In case of experiments with gelatin substrate a stop watch was started simultaneously with the shaking and 2 ml. of the mixture was pipetted out immediately into a dry Ostwald type of viscometer previously maintained at 37° C. in a thermostat. Viscometric readings were started using a second stop watch five minutes after the mixing of the enzyme solution and the substrate. During this time thermal equilibrium had already been attained in the viscometer. Readings were repeated every five minutes for about fifty minutes after which the fall in viscosity was very slow. Initial speed of fall of viscosity was calculated by extrapolating viscosity/time curves to zero time and also the per cent. fall in initial viscosity in 30 minutes was calculated. Finally α-amino nitrogen was estimated in 2 ml. of the mixture, using a micro Van Slyke apparatus, immediately after completion of the viscosity readings and again after incubating at 40° C. for a definite period. There is no measurable increase in α-amino nitrogen during the one hour or so which must elapse after mixing of the enzyme solution with the substrate and before an aliquot portion can be analysed in the Van Slyke apparatus.

When the substrate protein used was an albumin, then aliquot volumes were pipetted out immediately after mixing the substrate solution with the proteinase solution and analysed for (*a*) the amount of organic nitrogenous compounds not precipitated by boiling at the isoelectric point of the protein, (*b*) the amount not precipitated by mixing with an equal volume of 8% trichloracetic acid and (*c*) the free α-amino acids by estimation by the Van Slyke method. The three estimations were repeated after incubation at 40° C. for a definite length of time. Micro-Kjeldahl method was used for the estimation of nitrogen compounds.

pH Optima of the hydrolysis of gelatin by the proteinases of thermophilic bacteria

2 ml. each of the proteinase solution were mixed with 20 ml. each of 3% 'Bacto' gelatin solutions prepared at different pH in McIlvaine's citrate-phosphate buffer and viscometric determinations and estimation of increase in α-amino acids were carried out as described above. The results are summarised in Tables I, II and III below.

All the three proteinases therefore have their pH optima in the alkaline region, *e.g.*, at pH 8·1 to 8·3 for *Bacillus thermophilus*, pH 7·5 to 7·7 for *B. aerothermophilus* and pH 8·0 to 8·1 for *B. thermoacidurans*. In this respect, therefore, they resemble the tryptases.

TABLE I

Optimum pH of the proteinase of B. thermophilus

pH	Initial rate. % Fall in initial viscosity in first 5 minutes	% Fall in initial viscosity in 30 minutes	Increase in α-amino nitrogen, mgm./10 ml. in 48 hours
3·0	0·67	5·35	0·00
4·0	0·94	10·80	0·00
5·0	2·24	21·30	3·10
6·0	5·37	40·15	6·13
7·0	8·15	48·00	8·65
7·5	9·65	51·35	10·13
8·0	10·63	55·50	11·67
8·5	10·50	54·60	11·68
9·0	10·27	51·25	10·15
9·5	10·00	51·00	9·40
10·0	7·28	46·50	8·15

TABLE II

Optimum pH of the proteinase of B. ærothermophilus

pH	Initial rate. % Fall in initial viscosity in first 5 minutes	% Fall in initial viscosity in 30 minutes	Increase in α-amino nitrogen, mgm./10 ml. in 48 hours
3·0	1·85	2·25	0·00
4·0	3·10	4·10	1·15
5·0	5·65	23·75	3·65
6·0	7·55	45·10	6·41
7·0	9·15	55·90	8·98
7·5	12·10	59·15	9·53
8·0	11·50	56·45	9·00
8·5	10·00	55·40	8·75
9·0	7·55	49·15	7·15
9·5	4·25	44·00	6·37
10·0	3·40	32·50	4·66

TABLE III

Optimum pH of the proteinase of B. thermoacidurans

pH	Initial rate. % Fall in initial viscosity in first 5 minutes	% Fall in initial viscosity in 30 minutes	Increase in α-amino nitrogen, mgm./10 ml. in 48 hours
3·0	1·68	1·95	0·00
4·0	2·15	3·00	0·00
5·0	3·17	19·65	3·15
6·0	5·68	32·70	5·01
7·0	7·86	44·00	6·65
7·5	9·79	48·35	8·22
8·0	11·53	50·65	8·68
8·5	9·61	49·30	8·25
9·0	9·38	47·45	8·00
9·5	7·95	44·65	6·41
10·0	5·40	30·40	4·78

Peptone splitting enzyme in filtrates from the cultures of the thermophilic bacteria

Besides the true proteinase the thermophilic bacteria also produce an enzyme which can hydrolyse peptone and which is distinct from the proteinase. The enzyme solutions used in these experiments were filtrates from four days' old cultures. 20 ml. of a 2% solution of Witte's peptone made up at different pH in McIlvaine's citrate-phosphate buffer were incubated at 40° C. for forty-eight hours with 2 ml. of the enzyme solution and the increase in α-amino acids was estimated by Van Slyke's method. The results are tabulated in Table IV and it is clear that the optimum pH of this enzyme is between 7·5 and 8·0.

TABLE IV

Optimum pH of bacterial peptonase

Increase in α-amino nitrogen mgm./10 ml. in 48 hours

pH	*B. thermophilus*	*B. aerothermophilus*	*B. thermoacidurans*
3	0·00	0·00	0·00
4	0·00	0·00	..
5	..	0·33	..
6	0·57	0·75	0·93
7	1·63	1·82	2·15
8	2·00	1·98	2·20
9	1·04	0·86	1·01
10	0·22	0·45	..

Course of formation of proteinase and peptonase in cultures of thermophilic bacteria

The course of increase or decrease of proteinase and of peptone splitting enzyme in cultures was followed daily during an incubation period of one week. As before culture filtrates served as the enzyme solutions. The results are summarised in Table V.

The polypeptidase capable of splitting peptone therefore first appears when the cultures are two or three days old and then goes on increasing steadily, whereas the proteinase is found in culture filtrates in appreciable quantity within twenty-four hours of inoculation and reaches a maximum in two or three days, after which the proteinase activity of the filtrates decreases gradually. Thus filtrates from thirty-six or forty-eight hours old cultures do not contain any enzyme capable of further hydrolysing the products obtained by proteolytic degradation of a true protein. Most previous investigators do not seem to have taken the precaution of ensuring that their enzyme extracts were free from peptonases and polypeptidases

TABLE V

Course of formation of proteinase and peptone splitting enzyme by bacteria

2 ml. of the culture filtrate to every 10 ml. of 3% 'Bacto' gelatin substrate or 10 ml. of 2% Witte's peptone substrate, both at optimum pH

Age of culture in hours	Initial rate. % Fall in initial viscosity in first 5 minutes	% Fall in initial viscosity in 30 minutes	Increase in α-amino nitrogen, *mgm.*/10 *ml. in* 48 *hours*	
			Proteinase	Peptonase
		B. thermophilus		
24	4·63	22·15	4·00	0·10
48	12·25	61·70	13·73	0·35
72	12·90	62·50	13·80	0·80
96	11·86	57·65	11·37	1·13
120	10·75	54·10	10·73	1·72
144	8·12	47·25	8·33	1·96
160	6·67	33·40	6·78	2·17
		B. aerothermophilus		
25	4·79	30·10	4·39	0·00
49	11·30	35·60	10·73	0·15
74	11·35	56·75	11·17	0·40
97	10·17	53·15	9·63	1·12
121	9·00	47·10	8·17	1·68
145	7·17	40·25	6·83	2·05
162	6·90	31·70	5·91	2·30
		B. thermoacidurans		
24	4·71	28·10	4·33	0·00
48	9·73	47·85	8·13	0·05
72	9·96	49·30	8·62	0·23
96	8·89	44·10	8·00	1·03
120	7·73	37·35	7·63	1·61
144	6·15	30·00	7·11	1·93
160	5·33	21·30	6·97	2·18

although the methods of analysis adopted by them depended solely on the estimation of increase of free α-amino acids. Maschmann (1937) has tried to eliminate the polypeptidase from his enzyme solutions by a laborious system of adsorption.

Hydrolysis of casein by the proteinases of the thermophilic bacteria

The substrate solutions were prepared at different pH in McIlvaine's buffer and contained generally 1·4 to 3·0 mgm. organic nitrogen per ml. Typical results obtained with casein are summarised in Table VI from which it is clear that casein is efficiently hydrolysed, the optimum pH being 7·3 to 7·7.

TABLE VI

Hydrolysis of casein by bacterial proteinases

Proteinase from	Increase in α amino-nitrogen, mgm./10 ml. in 48 hours				
pH ..	5	6	7·5	8·5	9·5
B. thermophilus	3·01	3·43	5·21	4·83	3·37
B. aerothermophilus	5·35	7·08	9·10	6·05	..
B. thermoacidurans	1·42	1·53	4·24	3·11	2·56

Hydrolysis of albumins by bacterial proteinases

In contrast to casein and gelatin, egg albumin is very resistant to proteolytic action of the proteinases under study. This is obvious from the data summarised in Tables VII, VIII and IX. The optimum hydrogen ion concentration appears to be at pH 7·5 to 8·0 approximately.

TABLE VII

Hydrolysis of albumin: B. thermophilus

pH	Increase in nitrogen not precipitated at isoelectric point, mgm./40 ml. in 48 hours	Increase in nitrogen not precipitated in 4% trichloracetic acid, mg./40 ml. in 48 hours	Increase in α-amino nitrogen, mgm./10 ml. in 48 hours
3	0·00	0·00	0·00
4	0·00	0·00	0·00
5	0·00	0·00	0·00
6	0·00	0·00	0·00
7	6·32	2·35	0·50
8	8·41	2·58	0·68
9	3·27	1·25	0·00
10	0·00	0·00	0·00

TABLE VIII

Hydrolysis of albumin: B. ærothermophilus

pH	Increase in nitrogen not precipitated at isoelectric point, mgm./40 ml. in 48 hours	Increase in nitrogen not precipitated in 4% trichloracetic acid, mgm./40 ml. in 48 hours	Increase in α-amino nitrogen, mgm./10 ml. in 48 hours
3	0·00	0·00	
4	0·00	0·00	
5	0·00	0·00	
6	1·32	0·00	Nil
7	3·86	1·37	
8	4·20	1·43	
9	1·05	0·00	
10	0·00	0·00	

TABLE IX

Hydrolysis of albumin: B. thermoacidurans

pH	Increase in nitrogen not precipitated at isoelectric point mgm./40 ml. in 48 hours	Increase in nitrogen not precipitated in 4% trichloracetic acid, mgm./40 ml. in 48 hours	Increase in α-amino nitrogen, mgm./10 ml. in 48 hours
3	0·00	0·00	
4	0·00	0·00	
5	0·00	0·00	
6	1·94	0·00	Nil
7	5·38	1·38	
8	5·93	2·11	
9	2·15	0·00	
10	0·00	0·0·	

Serum albumin and serum and the proteinases of thermophilic bacteria

Serum albumin was found to be even more resistant to hydrolysis by the proteinases of the thermophilic bacteria. Only very slight increase in organic nitrogen not precipitable by boiling at isoelectric point or by trichloracetic acid could be detected at all the hydrogen-ion concentrations studied. From this very slight increase it was impossible to find out the optimum pH of hydrolysis but it appeared to be somewhat above 7. Increase in α-amino nitrogen could not be detected. Data is not cited here as extent of hydrolysis was very meagre. Incidentally it was found that not merely serum albumin is resistant to attack itself but also that liquid serum strongly inhibits the activity of the proteinases here studied. As expected, denaturation of egg albumin and serum albumin rendered them more readily available for attack by these proteinases.

Relationship between time and percentage of hydrolysis

The bacterial proteinases studied here are fairly powerful and it was found that linear relationship was obtained by plotting per cent. fall in viscosity of gelatin solution against the square root of time only. This is illustrated for *Bacillus thermophilus* proteinase in Fig. 1. The curves in case of the proteinases of *B. aerothermophilus* and *B. thermoacidurans* are quite similar.

Relationship between percentage of hydrolysis and enzyme concentration

On the basis of the Michaelis and Menton's theory it would be expected that velocity of hydrolysis will vary directly with the enzyme concentration. Varying results have been obtained regarding relationship of percentage of

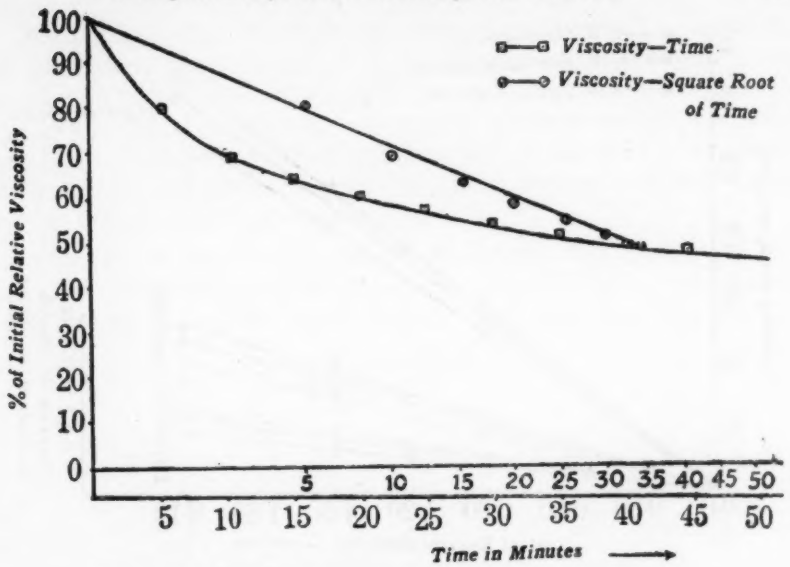

FIG. 1. Velocity/Time Curves for Proteinase of B. thermophilus

hydrolysis to enzyme concentration by different workers in case of trypsin. [See for example Bergmann and Pojarlieff (1934); Bergmann and Föhr (1933) and Farber and Wynne (1935).]

In case of pepsin, Anson and Mirsky (1932) have found direct relationship between the rate of hydrolysis and enzyme concentration only when enzyme and substrate concentrations were low. Direct relationship has been found for gelatin hydrolysis in the case of malt proteinases by Luer and Malsch (1929). In the case of proteinases of the thermophilic bacteria here studied linear relationship was obtained irrespective of whether the initial rate of hydrolysis or the progressive rate as represented by the per cent fall in initial viscosity of gelatin solutions in thirty minutes or measurement of the amount of free amino acids set free is used as the basis. This is shown graphically in Fig. 2.

In 1913 Michaelis and Menton announced that enzymic processes are preceded by a combination of the enzyme molecule and the substrate molecule. Evidence that has later on been accumulated by some other workers has generally supported this view. Kinetics of enzymic reactions can be satisfactorily interpreted on this basis. Further evidence in support of this view is afforded by the fact that many enzymes are protected by their substrates against heat. For example, the thermostability of the microbial enzymes here studied is greater in presence of proteins and peptone than in their

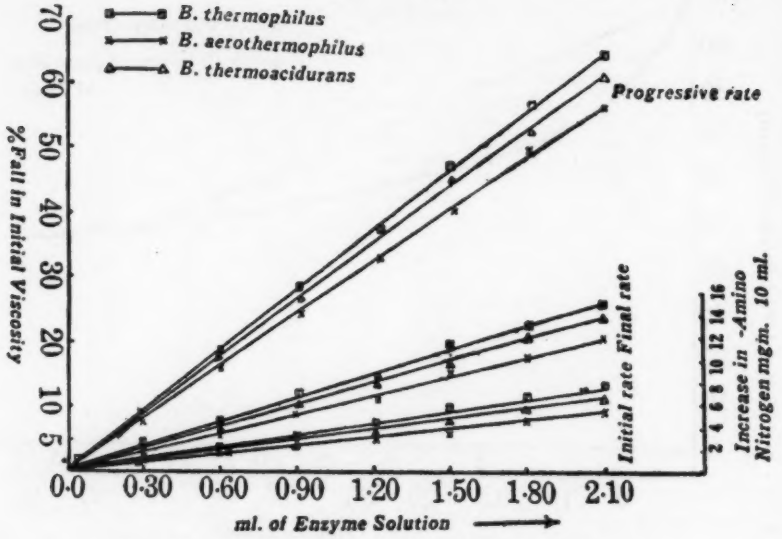
B. thermophilus
B. aerothermophilus
B. thermoacidurans
Progressive rate
Initial rate Final rate
% Fall in Initial Viscosity
70 60 50 40 30 20 10 5
0·0 0·30 0·60 0·90 1·20 1·50 1·80 2·10
ml. of Enzyme Solution
2 4 6 8 10 12 14 16
Increase in -Amino Nitrogen mgm. 10 ml.

FIG. 2. Enzyme Concentration and Hydrolysis

absence and the destruction of activity of trypsin solution which takes place rapidly at room temperatures is prevented by the addition of a very small quantity of gelatin or peptone. Data need not be cited here, but the effects are quite striking. At least in one case, Stern (1936), formation of the intermediate enzyme-substrate complex has been proved by spectrum analysis. According to Northrop (1932), however, trypsin does not form a compound with its substrate. More recent work by Farber and Wynne (1935) has, on the other hand, proved that Michaelis and Menton's equation is in fact applicable.

In the present experiments same quantity of enzyme was added to gelatin substrates of different concentrations varying from 1·2 to 4%. The data are set forth in Table X.

TABLE X

Velocity of hydrolysis and substrate concentration

Proteinase of	% Fall in initial viscosity in 30 minutes at substrate concentration of				
	1·2%	1·9%	2·6%	3·3%	4%
B. thermophilus	..	26·20	33·35	37·60	44·15
B. aerothermophilus	18·75	26·95	33·10	35·00	39·05
B. thermoacidurans	16·30	15·80	21·20	20·50	..

On equations based on Michaelis and Menton's theory, Linweaver and Burk (1934) have shown that the plot of the ratio of initial substrate concentration to reaction rate (S/V) against the initial substrate concentration (S) yields a straight line for the case in which an enzyme-substrate compound consisting of one molecule each of the enzyme and the substrate is postulated. This method of plotting has also been employed by Greenberg and Winnick (1940) in case of certain plant proteases.

In the case of microbial proteinases here studied S/V plotted against S gave straight line curves where V was equal to per cent fall in initial viscosity in first five minutes or first thirty minutes. This is evident from Fig. 3.

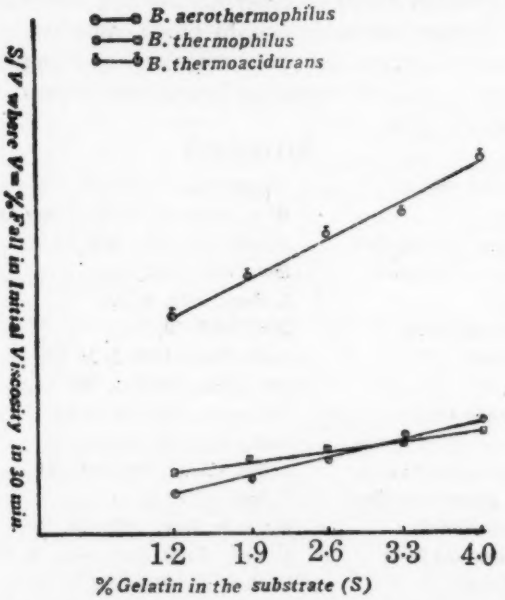

FIG. 3. Substrate concentration against ratio of Substrate Concentration and Viscosity

From these results it appears that with these bacterial proteinases the enzyme combines with the substrate before hydrolysing it and that the enzyme-substrate complex consists of one molecule each of the enzyme and the substrate.

SUMMARY

1. The thermophilic bacteria *Bacillus thermophilus, B. aerothermophilus* and *B. thermoacidurans* produce powerful proteinases which can be detected in the culture filtrates.

2. These proteinases resemble tryptases in their optimum hydrogen-ion requirements and hydrolyse gelatin and casein readily and albumins sparingly, unless the albumin has been previously denatured.

3. In addition to the proteinases these thermophilic bacteria also produce a polypeptidase capable of hydrolysing peptone, but this enzyme appears in culture filtrates much later than the proteinases.

4. Velocity of gelatin hydrolysis by these proteinases varies directly as the square root of time and percentage of hydrolysis varies directly with the enzyme concentration.

5. Relationship of substrate concentration and rate of hydrolysis shows that in case of gelatin Michaelis and Menton's equation is applicable and that an intermediate enzyme-substrate complex consisting of one molecule each of the enzyme and the substrate is probably formed before the substrate is hydrolysed.

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STUDIES ON INDIAN BANANAS

I. A Descriptive Study of Twenty-four Varieties*

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The specific and varietal relations as well as the classification of the banana, an important cultivated agricultural crop, is ill-defined and in some cases contradictory (Linnaeus, 1753; Baker, 1893; Schumann, 1900; Jacob, 1934 and others). The chief reason for the existing confusion in the taxonomy of the cultivated bananas appears to be the lack of proper descriptive study of the numerous varieties, both cultivated and wild. The importance of a detailed descriptive study is stressed by Teodoro (1915) and he is supported by Backer (1924) and Cheesman (1934). In his excellent discourse on the banana varietal problem, Cheesman (1934) discusses the numerous defects in the existing nomenclature and classification and stresses the importance of varietal descriptions suitably illustrated as a preliminary necessity for the study of the various varieties extant in each region before the existing chaos in nomenclature and classification can be removed.

Published work on South Indian bananas have not adequately satisfied the detailed study necessary for ready spotting out of well-known varieties of bananas (Jogiraju, 1931; Jacob, 1934, 1942 *a*, 1942 *b*). In this paper, therefore, an attempt has been made to thoroughly go into the question of the description of twenty-four well-known varieties of bananas grown in this province. This attempt has necessarily to be followed by other investigations in the lines undertaken here, which, in the opinion of the author, will put the banana classification on practical lines.

The materials for the study were obtained from the Banana Experimental Area and the College Orchards of the Agricultural Research Institute at Coimbatore. The brief descriptions presented in this paper are the sum effect of the bulky data collected during two years of investigation on a purely descriptive systematic work.

Description of Varieties

The names applied to the varieties in this paper are those standardised by Jacob (1934). To make the descriptions complete a few of the important

* This forms part of the thesis submitted for the M.Sc. degree of the Madras University.

synonyms of each variety are given, the synonyms referred to being extracts from *South Indian Bananas*, by Jacob (1934).

1. *Kullan*

Plant.—The plant is stout and dwarf attaining a height of about seven feet; trunk upto 25 inches in girth at base, light green in colour, tapers very little from the base to the crown; outer sheaths light green with dark spots at the junction of the leaf-sheath and petiole. Leaf-blades about 5 feet long and 24 inches wide at the widest part, green, glaucous on the under-surface, texture tough or leathery. Lamina base equal, deeply cordate. Petiole rather short and stout, margins winged and the channel wide; shape of leaf elliptic. Total number of leaves produced being about 29, and suckers 5. Duration medium (about 15 months).

Bunch.—Peduncle or stem thick, tomentose, almost at right angles to the pseudo-stem. Hands and fruits compact; fruits at right angles to the main axis; number of hands 8 to 12; the bunch may weigh from 25 to 30 lb.; the bottommost hand with about 20 fruits and the rest with 12 to 14 fingers or fruits arranged in two rows. The sterile axis is clothed with persistent male flowers and dried bracts.

Fruit.—Small, 3 to 4 inches long, about 4·5 inches in girth at the middle, plumpy, irregularly five sided and the angle ridges rather indistinct at full maturity; base broad, apex blunt; skin thin with little spongy fibre on the inner surface, leathery and bright yellow when fully ripened; pulp slightly tending to be yellow, juicy and sweet. Keeping quality of fruit good. This serves as a dual purpose variety (Plate V, Fig. 6; Text-Fig. 11).

Synonyms.—Rasalu or Rasa arati of Peddapur (East Godavari Dist.), Theer vazhai of Erode; Kuzhi vazhai of Udamalpet; Enugu arati of Jeypore.

2. *Nendrapadathi*

Plant.—Tall, attaining a height of about 10 feet; trunk about 24 inches in girth at base, tapers from base to crown; colour of pseudo-stem light green. Leaf blade about 7 feet long and 22 to 24 inches wide, green, leathery. Lamina base unequal, acuminate. Petiole long, margins pink coloured and slightly apart; total number of leaves 27; suckers 8 per plant. Duration medium (about 17 months).

Bunch.—Peduncle or stem tomentose, pendulous. Hands and fruits compact; fruits slightly curved; number of hands 8 to 10 and with about 14 fingers or fruits per hand. Bunch weighs about 40 lb. Male flowers persistent clothing the sterile axis.

Fruit.—Of medium size, 6 to 8 inches long, about 5·5 inches in girth at the middle, tapers to a distinct apex; five sided and the ridges prominent. Skin thick, leathery, lemon coloured, easily peels off from the pulp; pulp firm, cream coloured, core not fairly developed, flavour mildly pleasant, taste sweet with little acidity, seeds absent. Keeping quality fairly good. This is a dual purpose variety (Plate V, Fig. 8; Text-Fig. 5).

Synonyms.—Nendran vannan of Trichur Farm; Kuthiravalan of Pulamanthol (Malabar).

3. *Nana Nendran*

Plant.—The plant attains a height of about 10 feet, trunk about 22 inches in girth at base. Pseudostem light green with pinkish tinge in colour; tapers from base to crown. Leaf green, about 7 feet long and about 24 inches in width at the widest part; the margins of the petiole and the mid-rib of leaf coloured pink; petiole margins not wide apart but overlapping. Duration early maturing (about 14 months).

Bunch.—Drooping, stem thick but not clothed with hairs. Bunch not compact; number of hands 4 or 5 with about 7 to 9 long fruits per hand, fruit at almost right angles to the main axis. Bunch weighs about 15 lb. Male flowers and bracts persistent (Plate IV, Fig. 7).

Fruit.—Long, cylindrical, 11 to 12 inches in length, about 5 inches in girth, almost straight, irregularly five sided, ridges disappearing at full maturity; base narrowing; apex short but distinct; skin thick and leathery, with little spongy fibre on the inner surface; skin tough, golden yellow coloured, peeling off with difficulty from the pulp; pulp firm, slightly pink in colour, core fairly conspicuous, flavour mildly pleasant, taste medium sweet, quality medium as a dessert fruit; keeping quality good (Text-Fig. 13).

Synonyms.—Nendra vazha of Calicut; Nendra bale of Moodbidri (South Kanara); Thiruvodan of Tellicherry; Chengazhikodan of Trichur Farm; Ethakka of Alwaye.

4. *Attu Nendran*

Plant.—Tall, height about 11 feet, girth of trunk at base about 27 inches. Other characters same as Nana Nendran; number of leaves about 36, suckers 14. Duration late maturing (about 19 months).

Bunch.—Drooping, compact with 7 to 8 hands with long fruits, curved upwards. Medium bunch weighs about 30 lb. Male flowers and bracts persistent clothing the axis (Plate V, Fig. 1).

Fruit.—Long, slightly curved, pedicel curved, length about 9 inches, girth at middle about 5·5 inches; irregularly five sided, angle ridges distinct, sides slightly inflated, base narrowing, apex tapering with a prominent long pointed beak; skin thick with little spongy fibre on the inner surface, tough, golden yellow coloured when fully ripened, peels off with some difficulty from the pulp. Pulp firm, pinkish, core conspicuous, flavour mild, tastes as Nana Nendran. Good for culinary purpose (Text-Fig. 1).

Synonyms.—Nendran of Tellicherry; Nedu Nendran of Trichur Farm; Yethan of Sendamarai.

5. *Myndoli*

Plant.—Description same as that of Attu Nendran; leaves 34; suckers 16. Duration late maturing (about 19 months).

Bunch.—Same as Attu Nendran, but number of hands more than that of Attu Nendran; hands about 10, compact, fruits long and curved. Medium bunch weighs about 50 lb.

Fruit.—Same as Attu Nendran for all details.

6. *Moongil*

Plant.—Description same as that of Attu Nendran and Myndoli. Number of leaves about 35; total number of suckers about 17. Duration very late maturing (about 17 months).

Bunch.—Semi-erect, peduncle green, glabrous and thick; number of hands one or two, each with about 15 fruits; fruits curved showing the appearance of curved horns, and the axis ends blindly. Staminate flowers are not at all produced. Medium bunch weighs about 20 lb. (Plate V, Fig. 9).

Fruit.—Large, about 13 inches long, about 6·5 inches in girth at middle, cylindrical, curved; pedicel long and slightly twisted, irregularly five-sided angle ridges distinct, sides some flat and others slightly inflated; base narrowing, apex tapering with a prominent long pointed beak; skin medium thick with little spongy fibre on the inner surface, tough, golden yellow coloured, peeling off with some difficulty from the pulp; pulp firm, pink in colour, core fairly distinct, flavour mildly pleasant, taste medium sweet, quality medium as a dessert fruit, keeping quality good. Good for culinary purpose (Text-Fig. 8).

Synonyms.—Otta Mukil of Trichur Farm, Cochin State.

7. *Pacha Nadan*

Plant.—Attains a height of about 10 feet, about 25 inches in girth at base; pseudo-stem light green in colour, tapers from base to crown; leaf blades about 6·5 feet long and about 27 inches wide at the widest portion, green in colour, glaucous below, texture leathery, lamina base equal; petiole margins wide apart. Shape of leaf oblong; total number of leaves about 28, suckers about 9 per plant. Duration medium (about 15 months).

Bunch.—Drooping; peduncle or stem green, thick and glabrous; hands and fruits compact; fruits almost at right angles to the main axis. Number of hands 6 to 7 with about 12 fruits per hand. Medium bunch weighs about 25 lb. Sterile axis naked, *i.e.*, the staminate flowers and bracts are deciduous, and the axis ends in a pendulous 'heart' (Plate IV, Fig. 9).

Fruit.—About 5·5 inches long; 4·5 inches in girth at the middle, almost straight and uniform in cross-section, irregularly 5-angled, angle ridges prominent, sides rather flat; apex blunt and not prominent. Skin greenish yellow, peeling off easily from the pulp, with a large amount of spongy fibre on the inner surface; pulp firm, rather white in colour, core not conspicuous, flavour mild, taste rather saltish, quality medium to fair keeping quality poor. The fruit is used both when green and ripe (Text-Fig. 12).

Synonyms.—Eradan and Thodan of Perintalmanna (Malabar); Kadali of Mayavaram; Kali of Tanjore; Pacha Ladan of Trichy; Pachai Vazhai and Kali of Pollachi; Kal Kadali of Srivilliputur; Pacha Bale of Hospet; Pacha arati of Cumbum (Kurnool District).

8. *Kali*

Plant.—Description the same as that of Pacha Nadan. Duration early (about 13 months).

Bunch.—Almost same as that of Pacha Nadan; the bunch weighs about 20 lb. Sterile axis pendulous and naked.

Fruit.—Medium to small, about 4·5 inches long and about 4·5 inches in girth, spindle shaped, almost straight, stout and plump in the middle, somewhat 5-angled, angle ridges disappearing on full ripening, sides slightly inflated, base broad, tapering to a short pedicel; apex broad, blunt with a short and thick beak; skin thick, leathery to tough with a considerable amount of spongy fibre on the inner surface, lemon yellow coloured when fully ripened, peeling off easily from the pulp; pulp firm, cream coloured, core slightly conspicuous; taste sweet but little acidic, flavour pleasant; quality fair to good (Plate V, Fig. 4; Text-Fig. 4).

Synonyms.—Vannan of Kongad; Padathi of Alwaye; Mannan of Mannantoddy; Vellai Chingan of Sankarankoil; Pacha nadan of Thisayanvilai; Kuppamannan of Nileshwar; Valiyathodan of Nilambur; Mundilapadan of Nilambur; Kali Thodan of Nilambur; Kai Vazhai, Pakki, Padu Vazhai and Vella Padan of Gudalur (Nilgiris).

9. *Kunnan*

Plant.—Pseudo-stem slender as compared with the other varieties. Height of plant about 9 feet, girth at base about 20 inches; colour of trunk light green. Leaf narrow, 7 feet long and 22 inches wide; lamina thin and soft; leaves erect, petioles slender and long; lamina base equal; petiole margins touching. Duration medium (about 16 months).

Bunch.—Small, compact and pendulous; hands and fruits compact, slightly turned upwards. Number of hands about 10 with about 12 fingers to a hand. Medium bunch weighs about 20 lb. Sterile axis naked and pendulous (Plate IV, Fig. 2).

Fruit.—Small, length about 4·5 inches, and about 4·5 inches in girth at middle, slightly ridged, and the angle ridges absent when fully ripened. Pedicel distinct; apex prominent and pointed. Skin, green and shiny, thin with a slight amount of spongy fibre on the inner surface, bright yellow when ripe, and easily peels off from the pulp; pulp cream-coloured, firm, when fully ripened, sweet with an aroma. Keeping quality good. This is a dual purpose variety (Text-Fig. 6).

Synonyms.—Jirike Bale of Kallamandkur (S. Kanara); Tirunolli Kadali of Kasaragod (S. Kanara); Adukku Poovan of Nileshwar; Adukkan of Tellicherry; Madras arati of Piridi near Bobbili; Vellai Kadali of Sankarankoil; Nar Kadali of Sendamaram; Sanna Akulu chettu of Nidubrolu (Guntur District).

10. *Adakka Kunnan*

Plant.—Same as that of Kunnan.

Bunch.—Compact with a large number of fruits; number of hands 9 to 10, with 12 fruits per hand. Medium bunch weighs about 20 lb. Sterile axis mostly naked (in some cases a few clusters of dried male flowers were observed to be persistent on the axis) and pendulous (Plate IV, Fig. 3).

Fruit.—Small, about 4 inches long and about 5·5 inches in girth at the middle; spindle-shaped, plumpy at the middle, ridges absent; base broad, apex prominent with a pointed beak; skin green with an ashy bloom, thin, turns dull yellow when completely ripe, peels off from the pulp with some

difficulty; pulp firm, cream coloured, core distinct, sweet, keeping quality fairly good. Green fruits used for culinary purpose (flour made out of this is considered an ideal infant food) (Text-Fig. 7).

Synonyms.—Chara Kunnan of Kongad (Malabar); Veneetu Kunnan of Perintalmanna; Mutti Kunnan of Perinthalmanna (Malabar); Mundi Kunnan, Vennir Kunnan of Pulamanthol; Cheru Kunnan of Trichur Farm.

11. *Karim Kadali*

Plant.—Tall, about 11 feet high; 22 inches in girth at base; pseudo-stem or trunk deep purple in colour with greenish tinge, tapers from base to crown. Leaf about 7 feet long and 27 inches wide at the widest part of the lamina; lamina green, mid-rib purple coloured both on the inner and outer surface, base unequal, left side longer than the right. Petiole long, margins wide apart, left margin winged and deep purple in colour; shape of leaf oblong; total number of leaves 29; total number of suckers 18. Duration medium (about 14 months).

Bunch.—Large, compact with 7 hands, each with 14 fruits, fruits all curved; peduncle stout, brownish green, tomentose. Sterile axis slightly purple, long, ending in a heart-shaped flower bud, bracts purple in colour. Medium bunch weighs about 25 lb. (Plate IV, Fig. 1).

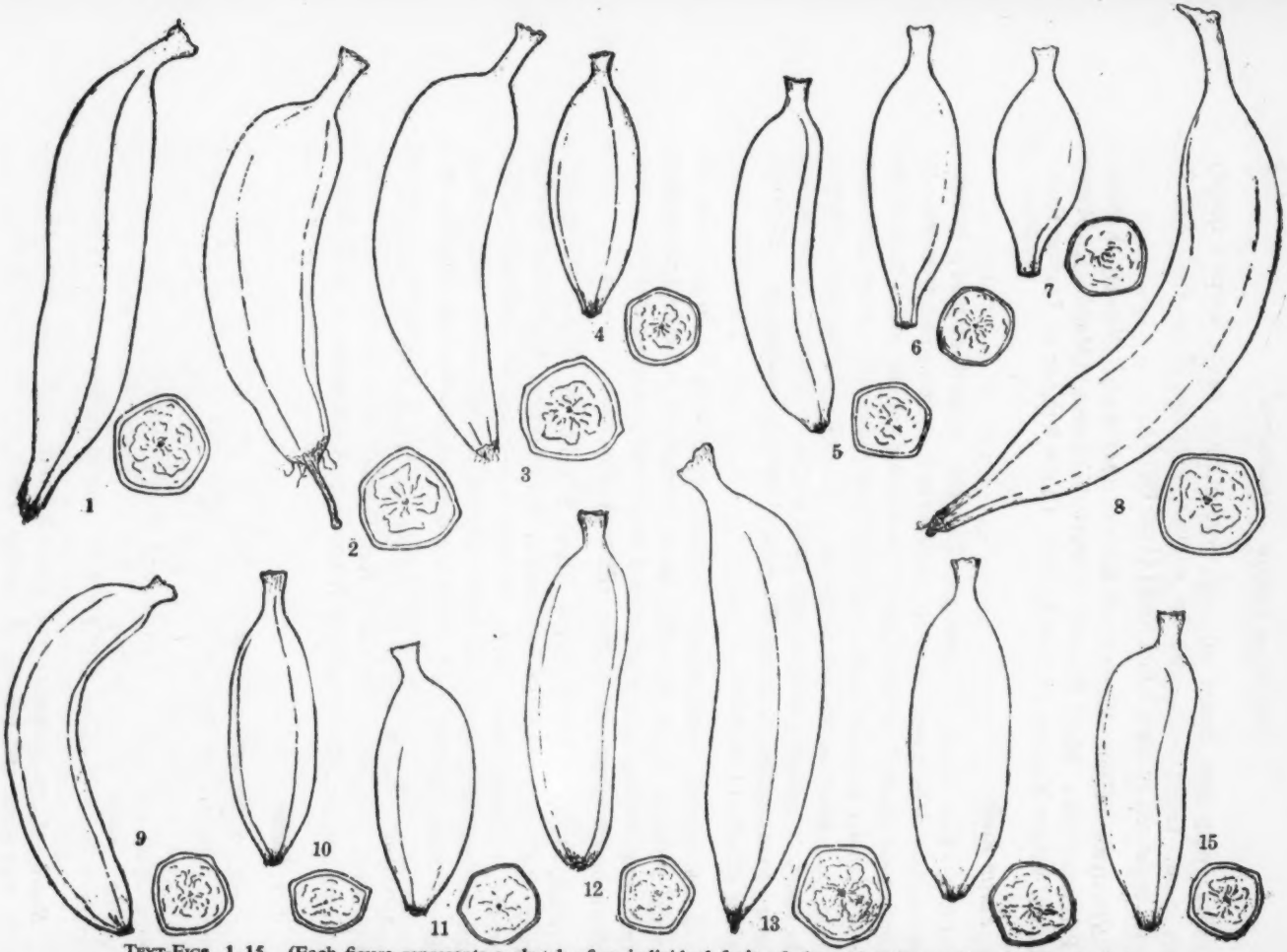
Fruit.—Long about 10 inches, about 5 inches in girth at the middle, curved, angle ridges five and indistinct at full maturity; base broad, pedicel short and indistinct; apex blunt, and beak rather absent; skin thick, yellow when ripe and easily peels off from the pulp; pulp firm, pale yellow coloured, sweet; quality good. This is a dual purpose variety (Text-Fig. 9).

Synonyms.—Karim Kadali of Alwaye and Trichur Farm; Irachikai of Kurumathur (Malabar), Irachiketti Kai, Chodari of Kuttuparamba; Vettan of Mannantoddy; Kari Vazha of Nilambur; Anaikomban of Kulithalai.

12. *Pey Ladan*

Plant.—Stout and tall; height about 12 feet; girth at base about 28 inches; pseudo-stem dark green, with slight pinkish tinge at base. Leaf long, length of lamina about 7 feet and width about 24·5 inches; base of lamina equal and cordate; petiole long, margins slightly pink coloured and touching. Shape of leaf oblong; total number of leaves about 40; total number of suckers about 22. Duration late maturing (about 19 months).

Bunch.—Large, compact with about 8 hands, each with about 14 fruits. Sterile axis stout and naked. A normal bunch weighs about 20 lb.



TEXT-FIGS. 1–15. (Each figure represents a sketch of an individual fruit of the variety in question ¼ natural size)

FIG. 1. *Attu Nendran.* FIG. 2. *Monthan.* FIG. 3. *Nalla Bontha.* FIG. 4. *Kali.* FIG. 5. *Nendrapadathi.* FIG. 6. *Kunnan.* FIG. 7. *Adakka Kunnan.* FIG. 8. *Moongil.* FIG. 9. *Karim Kadali.* FIG. 10. *Enna Benian.* FIG. 11. *Kullan.* FIG. 12. *Pacha Nadan.* FIG. 13. *Nana Nendran.* FIG. 14. *Nay Mannan* or *Venneettu Mannan.* FIG. 15. *Kurl Bontha.*

Fruit.—Short, flat sided, angle ridges fairly prominent; base broad and apex slightly tapering to a blunt beak, pedicel short; skin green with slight bloom and dull yellow when fully ripe, thick and leathery with a considerable amount of spongy fibre on the inner surface, peels off easily from the pulp; pulp rather white in colour, texture loose, core conspicuous, taste sourish sweet, quality not good as a dessert fruit.

Synonyms.—Mada Vazhai of Erode; Pey Monthan, Pey Valai of Trichy; Peyan of Coimbatore; Mayil Vazhai of Kuttanandal (Madura).

13. *Mayil Vazhai*

Plant.—Height about 9 feet; girth at base about 23 inches; pseudo-stem stout and dark green in colour; leaf long, about 6 feet and about 24 inches wide at the broadest portion of the lamina; lamina base equal, cordate. Petiole long and stout; petiole margins pink in colour, and almost touching each other. Shape of leaf oblong; total number of leaves per plant about 27, total number of suckers produced per plant about 17. Duration medium (about 15 months).

Bunch.—Small, hands and fruits not compact; number of hands 5 to 7, each hand with about 12 fruits; peduncle green and stout and smooth. Sterile axis naked; male flowers deciduous; a medium bunch weighs about 20 lb. (Plate IV, Fig. 8).

Fruit.—Small, angle ridges being prominent; base broad and ends in a short pedicel; apex blunt with an indistinct beak; skin green with a slight ashy bloom, dull yellow when ripe, thick and tough with a considerable amount of spongy fibre on the inner surface, easily peels off from the pulp; pulp white and soft, sweet but little acidic; quality medium as a dessert fruit; occasionally a few seeds are met with at the apical end—seeds small and of the shape and size of a black pepper.

14. *Ney Mannan*

Plant.—Tall, about 10 feet high, girth at base about 26 inches; pseudo-stem dark green in colour with an ashy bloom when young, leaf long, lamina about 6·5 feet long, width about 25 inches; lamina base equal, cordate; petiole long, margins pink in colour and almost touching each other; shape of leaf oblong; total number of leaves about 31, total number of suckers about 16. Duration medium (about 15 months).

Bunch.—Large, very compact with about 10 hands and about 145 fruits; peduncle green, glabrous and stout; sterile axis naked; male flowers deciduous; a normal bunch weighs about 45 lb.

FIG. 13. *Nana Nendran.* FIG. 14. *Ney Mannan or Vennettu Mannan.* FIG. 15. *Kari Bontha.*

Fruit.—Small to medium, 4·6 inches long, 4·3 inches in girth, ovate oblong, almost straight, plump at base and tapering at apex, irregularly five sided, angle ridges fairly distinct, sides flat to slightly inflated, base broad tapering abruptly to a medium angled pedicel; apex broadly tapering with a short thick beak; skin rather thick, leathery to tough with considerable amount o spongy fibre on the inner surface, peels off easily from the pulp, orange yellow in colour when ripe and dark green when unripe; pulp white in colour, firm, core inconspicuous, flavour pleasant, taste sweet, quality medium to good. A few seeds are occasionally met with; the green unripe fruits are generally used for culinary purposes (Plate V, Fig. 7; Text-Fig. 14).

Synonyms.—Thiyyan Mannan, Iyyan Mannan of Tellicherry; Mala Vannan, Kallu Vannan of Calicut; Ney Vannan of Kongad; Malamundi of Nilambur; Patinharan of Perinthalmanna; Nattu Vazhai of Pannakadu; Vayal Vazhai of Sankarankoil; Peyan of Nanguneri; Kari Bale of Ramapuram; Javari of Hospet; Bhynsi mouze of Kurnool, etc.

15. *Venneettu Mannan*

Plant.—Description same as for Ney Mannan.

Bunch.—Description same as that of Ney Mannan (Plate V, Fig. 2).

Fruit.—Same as that of Ney Mannan except that it is ashy coated when unripe, and dull yellow when ripe; green fruit used as a vegetable (Plate V, Fig. 10).

Synonyms.—Sambal Vazhai of Malli; Peyan of Palamcottah; Boothi Bale of Ramapuram; Boothi Javari of Kampli; Boodithi of Kurnool, etc.

16. *Monthan*

Plant.—Tall, about 12 feet high, girth about 27 inches at base; pseudostem light green in colour; leaves large, lamina length about 6·5 feet, width at the broadest portion about 26 inches, lamina base equal and cordate; petiole long and stout, margins touching; total number of leaves about 32; total number of suckers about 16. Duration medium (about 15 months).

Bunch.—Not compact, with about 5 to 7 hands, each hand with about 10 fruits; sterile axis naked, male flowers deciduous; peduncle green and glabrous; a medium bunch weighs about 25 lb. (Plate IV, Fig. 4).

Fruit.—Large, about 10 inches long and 6 inches in girth at the middle; slightly curved, fairly plump, irregularly five-sided, angle ridges fairly prominent, sides flat or slightly inflated; base broad, tapring to a long, angled pedicel; apex broad with a prominent knob-shaped beak; skin thick, tough

to leathery with a considerable amount of spongy fibre on the inner surface, peels off with some difficulty from the pulp; skin straw yellow in colour when ripe; pulp firm, cream coloured, core fairly conspicuous, taste medium sweet, pasty when quite ripe, and quality poor as fresh fruit. Generally used for culinary purposes (Text-Fig. 2).

Synonyms.—Silandi of Moodbidri; Banga Bale of Udipi; Manga Bale of Kundapur; Aunda Bale of Mangalore; Mamga Kai of Kasaragode; Manga Vazha of Nileshwar; Sodari of Kurumathur; Thezhuthani of Tellicherry; Thenali of Calicut; Ponthan of Kongad; Ponnan Kaya of Perintalmanna; Ponnan of Nilambur; Chetti Kaya of Ponnani; Nathangi Monthan of Erode; Erode Monthan of Kulithalai; Kondai Monthan, Yendra Monthan of Trichy; Trichinopoly Monthan of Negapatam; Nattu Monthan of Tenkasi; Thoppul Vazhai of Thangachimatam; Kalyanakai of Hospet; Bontha of Kalava.

17. *Pacha Montha Bathees*

Plant.—Same as that of Monthan.

Bunch.—Very compact hands and fruits; number of hands more than five; sterile axis short and naked; medium bunch weighs about 40 lb.

Fruit.—Of varying sizes; the fruit of the first four or five hands are big in size as in Monthan, but the rest are short. Fairly plump, irregularly five sided, angle ridges fairly prominent, sides flat, base broad, tapering to a long angled pedicel; apex broad and blunt as in the Monthan. Other characters same as the Monthan.

Synonyms.—Batheesa of Gopalpur; Nalla Batheesa of Piridi; Pacha Batheesa of Palteru; Pacha Bontha Batheesa of Vizag.

18. *Sambrani Monthan*

Plant.—Same as in Monthan.

Bunch.—Same as in Monthan.

Fruit.—Same as Monthan, but the unripe fruit is ashy coated; the ripe fruit is dull yellow in colour.

Synonyms.—Bonya Kilandi of Udipi; Vennettu Manga of Nileshwar; Venneettan Thezhuthani of Mannantoddy; Boothan Kaya of Gudalur.

19. *Buditha Montha Bathees*

Plant.—Description same as that of Pacha Montha Bathees;

Bunch.—Same as Pacha Montha Bathees (Plate IV, Fig. 5).

Fruit.—Almost the same as Pacha Montha Bathees; skin of unripe fruit ashy coated, while that of a ripe fruit is dull yellow in colour.

Synonyms.—Thella Batheesa of Piridi; Boodithi Batheesa of Palteru.

20. *Nalla Bontha*

Plant.—Description same as that of Monthan.

Bunch.—Description same as that of Monthan (Plate V, Fig. 3).

Fruit.—Almost same as that of Monthan excepting that the apex in this case is not knob-shaped as in the Monthan, but it is sharp and pointed to an extent (Text-Fig. 3).

Synonyms.—Pacha Bontha of Gopalpur; Bontha of Panchadarla; Monthan of Tenkasi; Manattu Monthan of Sankarankoil; Palakolu Bontha of Jagarlamudi.

21. *Nalla Bontha Bathees*

Plant.—Description same as that of Nalla Bontha.

Bunch.—Description same as that of Pacha Montha Bathees.

Fruit.—Same as ordinary Monthan, excepting that the apex of the fruit is pointed and not knob-shaped.

Synonyms.—Isika Bontha, Pichika Bontha of Jagarlamudi.

22. *Thella Bontha*

Plant.—Almost the same as Nalla Bontha in all characters.

Bunch.—The same as in the Nalla Bontha.

Fruit.—Same as Nalla Bontha but the skin is ashy coated; when ripe the skin is dull yellow in colour.

Synonyms.—Boodithi Bontha of Gopalpur and Vizagpatam.

23. *Kuri Bontha*

Plant.—Height about 8·5 feet; girth at base about 25 inches; pseudo-stem dark green in colour; leaf-blade about 7 feet long and about 27 inches wide; petiole long, margins almost touching; total number of leaves about 33, and suckers about 16.

Bunch.—Not compact, small, hands about 8 with about 14 fruits or fingers per hand; medium bunch weighs about 20 lb.; sterile axis naked (Plate IV, Fig. 6).

Fruit.—Small, about 4·5 inches long and about 4·5 inches in girth at the middle, spindle shaped, almost straight, plump in the middle, nearly

five angled, angle ridges distinct, sides inflated; base tapering to a short pedicel; apex broadly pointed with a short and thick beak; skin thick, lemon coloured, leathery, peeling easily from the pulp; pulp slightly tough, almost white in colour, core indistinct, taste sourish sweet, slightly acidic, quality medium; seeds occasionally met with (Text-Fig. 15).

24. *Enna Benian*

Plant.—Height about 8·5 feet, girth at base about 24 inches; pseudostem light green in colour, with the margins slightly coloured pink and with a slight ashy bloom; length of lamina about 6 feet, width about 25 inches; petiole long, margins pink coloured and close together; lamina base equal and cordate; total number of leaves about 27, suckers about 23. Duration medium (about 15 months).

Bunch.—Small, not compact. Hands about six, each with about 14 fingers or fruits; medium bunch weighs about 15 lb.; sterile axis naked and long.

Fruit.—Medium sized, about 5 inches long and about 4·5 inches in girth at middle, spindle shaped, almost straight, somewhat irregularly five sided, sides flat, narrowing to base and tapering to apex with a short and thick beak; skin thick, leathery to tough, orange yellow coloured, with a considerable amount of spongy fibre on the inner surface of the skin; skin peels off easily from the pulp; pulp soft to firm, yellow coloured; core distinct, flavour mild, slightly acidic, quality medium as dessert fruit (Plate V, Fig. 5; Text-Fig. 10).

Discussion

The chaos caused by adopting different approaches for the study of nomenclature and classification of bananas has evoked criticism from a number of workers, notably by Cheesman (1934). The most important defect lies in the nomenclature of the varieties and this has been well emphasized by him in his excellent treatise on the banana varietal problem. The distribution of these edible bananas is so wide that the same variety is known by many names in the different regions where they are grown, hence the difficulty in standardising the varietal names becomes boundless. In India, Jacob (1934) has done considerable work to standardise the varietal names and has given a list of vernacular names to be used for the varieties, which really limits the application of his key to restricted areas of South India. Furthermore his descriptions of the varieties are by no means complete.

Linnaeus classified the different varieties under two species, namely, *Musa sapientum* and *Musa paradisiaca*, taking into consideration the deci-

duous or the persistent nature of the male flowers. Linnaeus's classification as pointed out by Cheesman (1934) cannot hold good in respect to some of the varieties at least, because the character of the perisistent or the deciduous nature of the male (functionally staminate) flowers has been shown by the above study to be a very variable character. For instance, a few plants of the variety Adakka Kunnan described in this paper were observed to have some clusters of the dried male flowers persistent on the axis. Again provision is not made to group those types which do not at all produce any staminate flowers. For instance, the variety Moongil described herein does not at all produce any male or staminate flowers and the fruiting axis ends blindly after the formation of one or two hands of fruits.

Confusion exists regarding the nomenclature of the species. Baker (1893) places '*paradisiaca*' as a sub-species of *Musa sapientum*, while Schumann (1900) considers '*sapientum*' as a sub-species of *Musa paradisiaca*, and the Kew Bulletin of 1894 lists '*paradisiaca*' as a variety of *Musa sapientum*. The widely grown dwarf banana is termed *Musa Cavendishii* Lamb., the dwarfness having been taken into consideration. The dwarfness happens to be so distinct in appearance that no confusion had hitherto arisen, but it has been shown by Cheesman (1933) that at least one tall banana variety appeared so closely related to the dwarf that if a specific name is to be used for the one it should also be applied to the other. And since the dwarf is linked by intergrading forms with more than one tall banana variety, the possibilities of confusion if *Musa Cavendishii* is to be admitted as a possible specific name, are boundless. Jacob (1934) classifies all the cultivated varieties of bananas even including the dwarf banana (*Musa Cavendishii*) under one species which he also names *Musa paradisiaca*.

It has already been stressed that no classification of the edible bananas is possible at all until much study has been devoted to the diagnosis of the varieties (Teodoro, 1915; Backer, 1924; Cheesman, 1934). The present study, though restricted to only a few varieties, has clearly brought out the futility of certain systems of classification and the importance of a descriptive study. The extant literature on banana descriptions is generally restricted to the study of the characters which are not always important from the viewpoint of the grower. Such features are inevitable when botanical considerations outweigh those of practical utility, and when living specimens are not invariably studied. In South India the leaf production in banana is a very important economic character while the production of suckers is of equal importance to the grower. The difference between the varieties is so great that it is a surprise that these characters are ignored in banana descriptions.

An important feature of the present study is to accord due importance to such economic characters.

Summary

1. Twenty-four varieties of bananas grown in South India, the fruit of which is either used for culinary purpose or both as a vegetable and a fruit, were chosen for the study.

2. The plants were studied from the time of planting the sucker in the field upto the time of the harvest of the fruit bunch, and a description embodying certain important characters is given for each variety.

3. The experimental data presented clearly indicates that in the classification of bananas it is of paramount importance to take the total effect of all the important characters, namely, the different plant characters such as the stem, leaf and fruit, with accurately drawn illustrations rather than depend on a minor character like the persistent or deciduous nature of the male flowers in the bunches.

Acknowledgements

The author gratefully acknowledges the kind help and encouragement he received during the course of the study from Sri. K. C. Naik, M.Sc. (Bristol), Fruit Specialist to the Government of Madras, who suggested the problem and under whose guidance the investigation was taken up. The author's thanks are also due to Sri. S. N. Chandrasekhara Iyer and Sri. K. C. Jacob of the Botany Section of the Madras Agricultural Department for their kindly permitting him to make use of some of their photographs of the bananas.

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Explanation of Plates

Plate IV

(This plate shows the fruiting bunches of nine varieties of bananas.)

Fig. 1. *Karim Kadali*—Note the naked axis after the fruiting region.

Fig. 2. *Kunnan.*

Fig. 3. *Adakka Kunnan*—Note the compact nature of the bunch.

Fig. 4. *Monthan.*

Fig. 5. *Buditha Montha Bathees*—Note the compact nature of the bunch and also the numerous hands of fruits.

Fig. 6. *Kuri Bontha.*

Fig. 7. *Nana Nendran*—Note the few number of hands and the persistent and dried bracts.

Fig. 8. *Mayil Vazhai.*

Fig. 9. *Pacha Nadan.*

Plate V

(This plate shows the fruiting bunches of five varieties and a hand in each of five varieties.)

Fig. 1. *Attu Nendran*—Note the compact bunch and the persistent bracts and flowers.

Fig. 2. *Venneettu Mannan*—Note the ashy bloom on the fruit.

Fig. 3. *Nalla Bontha.*

Fig. 4. A hand of *Kali.*

Fig. 5. A hand of *Enna Benian.*

Fig. 6. *Kullan*—Note the persistent bracts after the fruiting hands.

Fig. 7. A hand of *Ney Mannan.*

Fig. 8. A hand of *Nendrapadathi.*

Fig. 9. *Moongil*—Note the long and curved fruits, and the very short axis ending blindly without any flower or bract.

Fig. 10. A hand of *Venneettu Mannan*—Note the ashy bloom on the fruit.

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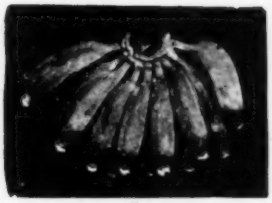

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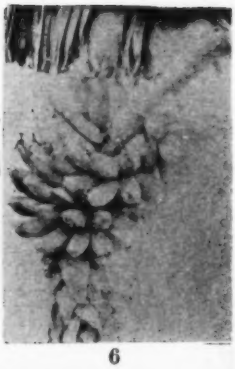

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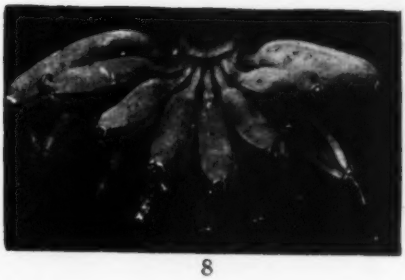

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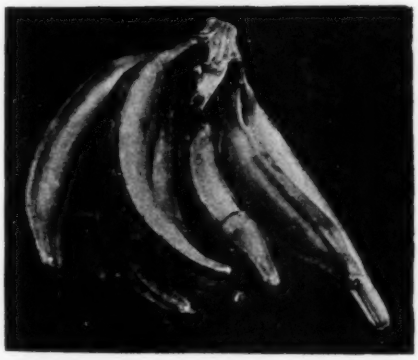

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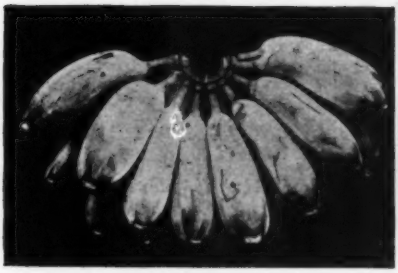

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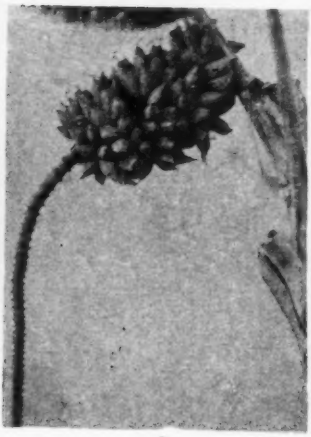

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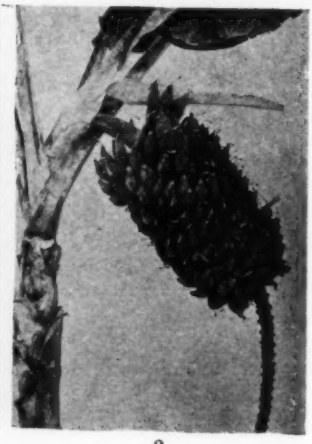

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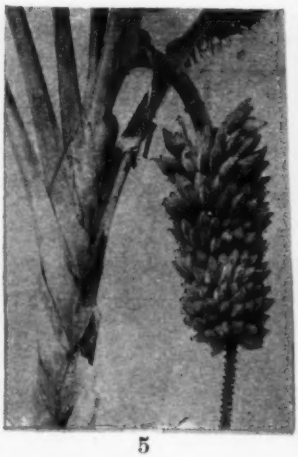

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THE ANATOMY OF THE REPTILIAN HEART

Part II. Serpentes, Testudinata and Loricata

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1. Introduction

In the first part of the present series the heart of *Varanus monitor* (Linn.), an example of the Sauria, was described in detail in order to serve as a basis for the investigation of the hearts of other reptiles. In the present part, the hearts of the Serpentes, Testudinata and Loricata are dealt with. The following species were selected for intensive study, the method of investigation being the same as in the case of *Varanus monitor*.

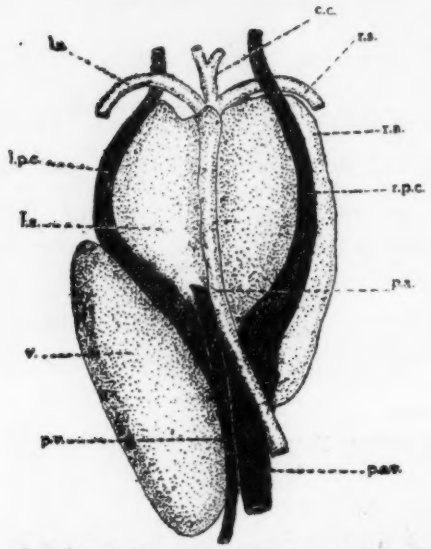

FIG. 1. The heart of *Natrix piscator* (Dorsal View). *c.c.*, the common carotid artery; *l.a.*, the left auricle; *l.p.c.*, the left precaval vein; *l.s.*, the left systemic artery; *p.a.*, the pulmonary trunk; *p.c.v.*, the post-caval vein; *p.v.*, the pulmonary vein; *r.a.*, the right auricle; *r.p.c.*, the right precaval vein; *r.s.*, the right systemic artery; *v.*, ventricle.

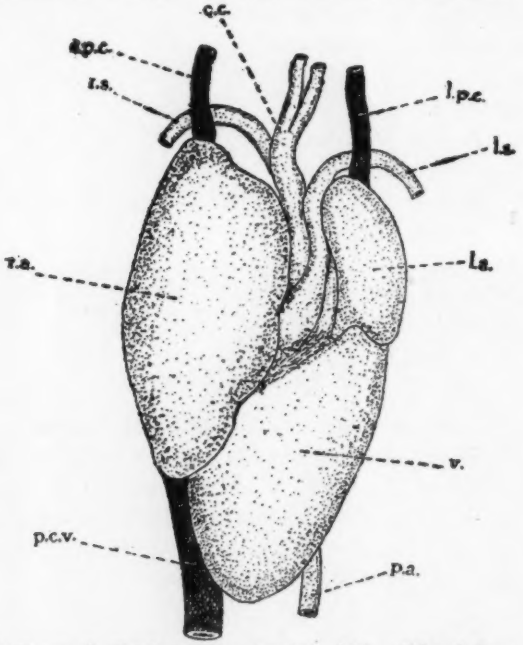

FIG. 2. The heart of *Natrix piscator* (ventral view). Abbreviations as in the previous figure.

1. Order ***Squamata***:
   Suborder ***Serpentes***: ***Natrix piscator*** (Schn.): 12 specimens.
2. Order ***Testudinata***:
   Lissemys punctata (Bonnaterre): 12 specimens.
3. Order ***Loricata***:
   Crocodilus palustris Lesson: 3 specimens.

Specimens of ***Natrix piscator*** and ***Lissemys punctata*** were collected in the suburbs of Agra, while a number of hearts of ***Crocodilus palustris*** were presented to me by Prof. Beni Charan Mahendra from his personal collection. A number of snakes (***e.g., Python molurus, Eryx johni, E. conicus,*** etc.) and some specimens of ***Gavialis gangeticus*** were used for confirmation and comparison.

As a comprehensive historical résumé and bibliography has already been given (Mathur, 1944), it is enough in the present part to refer to the relevant literature in the text and to give a list of the literature cited at the end.

II. The Heart of the Serpentes

(*a*) *General*

As the general structure of the ophidian heart has already been described by several workers, ***e.g.***, O'Donoghue (1912) in *Tropidonotus natrix*, Rau (1924) in ***Eunectes murinus***, and Ray (1934) in ***Ptyas mocosus***, it is scarcely necessary to give a detailed account of the heart of ***Natrix piscator*** (Schn.). I shall, therefore, confine myself only to those features which, although distinguishing the heart of this order from that of all other reptiles, have so far escaped the attention of anatomists.

In form the ophidian heart is considerably elongate, being generally about twice as long as broad. It lies generally in the anterior part of the cœlom, between the body-wall on the one hand and the œsophagus on the other, although it differs in its relative position in various species within wide limits (Thomson, 1913).

Anatomically, perhaps, one of the most conspicuous features of teh ophidian heart is its far-reaching asymmetry (Figs. 1 and 2). The right auricle in most snakes is twice or almost twice as large as the left[1] and is nearly equal to the ventricle in its capacity. It extends farther back than in any other reptilian heart, covering a considerable part of the dorsal and ventral surfaces of the right side of the ventricle. The ventricle has its left

[1] Although such a disparity in the sizes of the auricles is the rule in ***Colubridae***, the Boid Snakes ***Eryx conicus*** (Schn.) and ***Eryx johni*** (Russ.) show almost equal right and left auricles.

side nearly twice as long as the right, and the *coronary sulcus*, when seen in a ventral view, appears to be directed obliquely backwards from its left end to its right. The apex of the ventricle is displaced rather to the right side of the mid-longitudinal axis, and consequently the *posterior vena cava*, unlike that in other reptiles, occupies a sub-median position. The *sinus venosus* has its right half somewhat more developed than the left. The arterial trunks take their origin not exactly at the middle line of the heart but rather towards the left.

(*b*) *Minute Anatomy*

Although the general structure of the heart in snakes is well known, its minute anatomy has not been hitherto studied in serial sections. Goodrich's observations (1919) are apparently based on thick hand-cut sections and Rau (1924) had access to only a single heart of *Eunectes murinus* which he used for a study of "its gross structure in general and the relations of the interventricular septum to the opening of the arterial arches in particular." The following account which supplements the previous knowledge considerably, is almost entirely based on a scrutiny of successive transverse and longitudinal sections prepared by me and on dissections performed under the binocular microscope.

1. *Sinus Venosus.*—In connection with the ophidian *sinus venosus* three facts, not hitherto pointed out, may be particularly mentioned.

First, the left precaval vein (Fig. 3) opens into the combined bases of the post-caval and the right precaval immediately posterior to, or almost at the level of, the hind end of the sinu-atrial aperture.

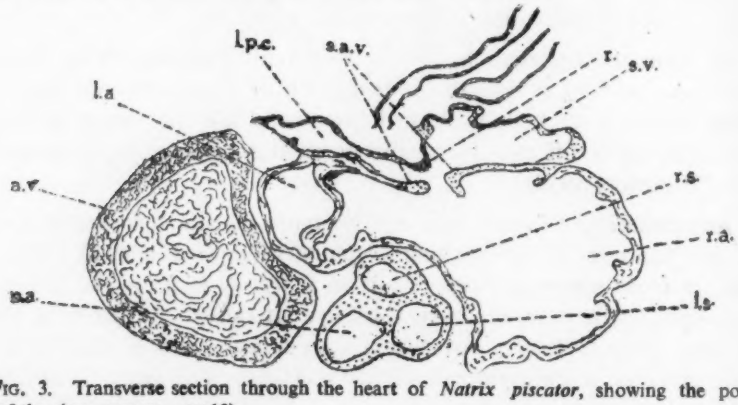

FIG. 3. Transverse section through the heart of *Natrix piscator*, showing the posterior part of the sinus venosus. (×10).

a.v., the anterior extension of the ventricle; *r.*, the ridge separating the bases of the post-caval and the right precaval vein from the base of the left precaval; *s.a.v*, the sinu-atrial valve; *s.v.*, sinus venosus. (Other abbreviations as in previous figures.)

Secondly, the base of the left precaval vein (Fig. 3) is separated from the combined bases of the post-caval and right pre-caval by a prominent ridge hanging downwards from the dorsal wall and extending considerably craniad to the place where the left precaval opens into the sinus venosus. This structure corresponds in its position neither to a ***tuberculum intervenosum*** nor to a ***septum sinu-venosi***.

Thirdly, the sinu-atrial aperture differs from that found in ***Varanus monitor*** in its form, position and direction. The ***suspending ligament*** and the ***sinu-atrial channel*** are absent and the sinus venosus, therefore, opens directly into the lumen of the right auricle. The ***sinu-atrial aperture*** (Fig. 3) is situated not on the dorso-lateral wall of the auricle as in ***Varanus monitor*** (Mathur, 1944), but almost in the middle of the dorsal surface. In all cases observed by me, the aperture is an ***elongated*** slit, not at right angles to the long axis of the heart, as reported for ***Eunectes murinus*** by Rau (1924), but disposed in a distinctly longitudinal direction. As a result of this, the sinu-atrial valves in snakes are situated not anterior and posterior to the aperture, but lateral to it.

2. ***Atrium Dextrum.***—The right auricle, as already mentioned, is much larger than the left. It projects backwards behind the anterior border of the ventricle, and just anterior to the point of emergence of the arterial trunks from the ventricle, is divided by a neck-shaped constriction into a small left and a large right lobe (Fig. 3). The constriction broadens out anteriorly (Fig. 4) so that the two lobes pass on insensibly into each other. The left lobe opens posteriorly into the ventricle (Fig. 5) almost at the level of the coronary sulcus through the atrio-ventricular aperture. The right one forms the posterior extension of the auricle, already mentioned in the general account of the heart and has the sinu-atrial opening in its dorsal wall to the right side of the constriction. There is no ***atrium dextrum intraventriculare*** in snakes.

3. ***Atrium Sinistrum.***—The left auricle is generally much smaller in size, and consequently in capacity, than the right. The quantity of aerated blood, therefore, entering the ventricle during the auricular systole is considerably less than that of the non-aerated.

Ray (1934) observed a prominent diverticulum at the inner antero-dorsal edge of the left auricle in ***Ptyas mucosus*** (Linn.) and compared it with a similar structure in the right auricle in ***Sphenodon punctatus*** (O'Donoghue, 1920), and ***Uromastix hardwicki*** (Bhatia, 1929). He thought that it might be due to " the constant pressure applied by the auricle on the underlined (sic.) left systemic arch." No such diverticulum was reported by O'Donoghue

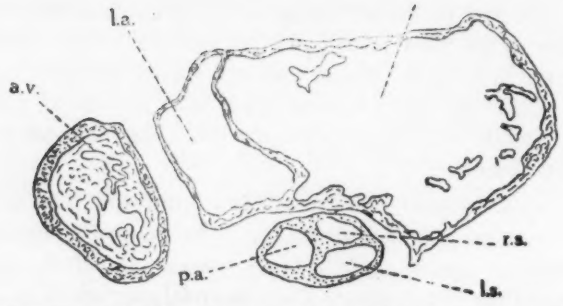

FIG. 4. Transverse section through the auricular region of the heart of *Natrix piscato-* × 10). (Abbreviations as in previous figures.)

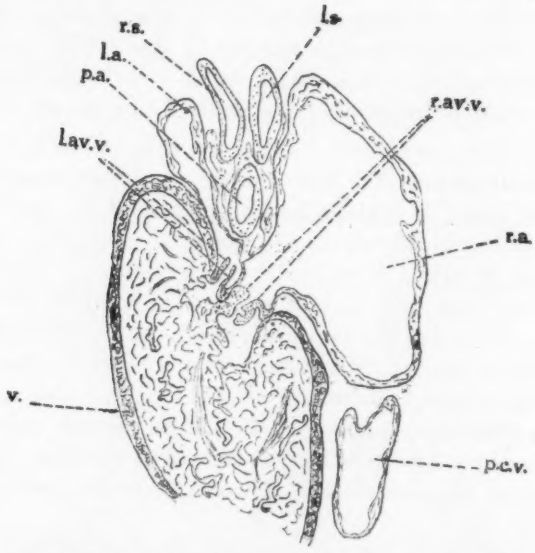

FIG. 5. Horizontal longitudinal section through the heart of *Natrix piscator* (× 8).
l.a. and *v.v.*, the left auriculo-ventricular valves; *r.av.v.* the right atrio-ventricular valves other abbreviations as in previous figures.

(1922) in *Tropidonotus natrix* and by Rau (1924) in *Eunectes murinus*. I have failed to find it also in *Python molurus* (Linn.), *Eryx conicus* (Schneid), *Eryx johni* (Russ.), *Lycodon aulicus* (Linn.), *Coluber diadema* Schleg. *Natrix piscator* (Schneid), and even specimens of *Ptyas mucosus* (Linn.) collected at Agra. The external configuration of such thin-walled chambers of the heart as the auricles and sinus venosus is considerably affected by the presence of coagulated blood, and it is as likely that the diverticulum described

by Ray (1934) might be due to such a cause as that it characterises the specimens of this species found in Bengal.

There is a great deal of difference amongst previous authors about the opening of the pulmonary vein. Fritsch (1869) pointed out that it is not guarded by a valve. Sabatier (1874) suggested that a fold of the auricle in this region acts as a valve during systole and prevents regurgitation,—a view which O'Donoghue (1912) regarded as highly probable and Rau (1924) confirmed in *Eunectes murinus*. Ray (1934), however, did not observe any such structure in *Ptyas mucosus*, and suggested that the inter-atrial septum in this region, abutting against the pulmonary aperture, acts as a temporary valve.

I feel that Ray's view is correct as in *Natrix piscator* the dorsal part of the inner-atrial septum (Fig. 6) forms a prominent fold exactly at the place

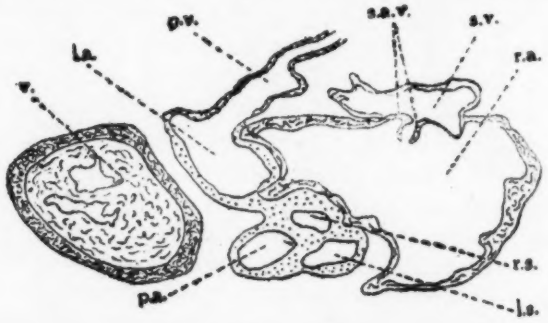

FIG. 6. Transverse section through the heart of *Natrix piscator*, showing the opening of the pulmonary vein (×10). Abbreviations as in previous figures.

where the pulmonary vein opens. Moreover, the terminal part of the pulmonary vein in *Natrix piscator* is abruptly dilated to form a small chamber opening into the left auricle. This chamber might supplement the action of the temporary valve formed by the atrial septum during the auricular systole, since it would act as a sort of backwater to check the backward thrust of the blood and thereby minimise the risk of regurgitation.

4. *Ventriculus and Arterial Trunks.*—The Ophidian Ventricle differs both externally and internally from that of other reptiles. It is considerably elongated, lies asymmetrically rather towards the left of the mid-longitudinal axis, projects forwards in front of and lateral to the posterior end of the left auricle, and accommodates the posterior part of the auricles in its anteromesial border which extends from the anterior end obliquely backwards towards the right. It is mainly on account of such a form and

Fig. 7. Transverse sections through the ventricle of *Natrix piscator* :—(A) through the apical region, (B) through the region of the muscular ridge, (C) just anterior to the free border of the muscular ridge, and (D) at the level where the auricles lead into the ventricle. (×10).

b.m.r., the base of the muscular ridge ; *c.d.*, cavum dorsale ; *c.p.*, cavum pulmonale ; *d.s.*, the dorsal septoid process ; *l.c.d.*, the left part of the cavum dorsale ; *p.r.a.*, the posterior part of the right auricle ; *p.v.l.a.*, the part of the ventricle receiving blood from the left auricle ; *r.c.d.*, the right part of the cavum dorsale ; *v.l.s.*, valves of the left systemic trunk ; *v.p.a.*, valves of the pulmonary trunk ; *v.s.*, the ventral septoid process (other abbreviations as in previous figures).

disposition that it shows a remarkable difference from that of other reptiles in its internal anatomy. Its features, as a whole, however, indicate a simplification of the saurian type.

In the ***apical region*** the ***horizontal septum*** is absent and there is thus no distinction between the ***cavum apicis dorsale*** and the ***cavum apicis ventrale***. Numerous well-developed trabeculæ (Fig. 7 A), however, are present, arranged more or less peripherally. These divide the internal space into a large number of lacunæ, inter-communicating with each other and opening into a central space which is compressed dorso-ventrally. A little in front of the posterior end in this region, a minute space separate from others may be noticed, situated ventro-laterally on the right side and leading forwards into the ***cavum pulmonale***. This is, ***sensu strictu***, homologous to the ***cavum apicis ventrale*** of ***Varanus monitor***.

With regard to the muscular ridge ("the interventricular septum") in the Ophidia, O'Donoghue (1918) observed that it "is very well developed and attached to the left dorso-lateral side of the ventricle, running from base to apex and directed towards the right ventro-lateral wall. Thus it runs in a direction entirely different from that of the septum in either Crocodilia or Chelonia—in fact, nearly at right angles to it—and divides the ventricular cavity, at any rate in systole, into a right dorso-lateral and a left ventro-lateral chamber." Goodrich (1919), however, reported that the disposition of the ridge was essentially similar in the hearts of ***Chelone***, ***Varanus*** and ***Python***, and Rau (1924) found that in ***Eunectes murinus*** "there projects into the cavity from the posterior apex and running dorso-ventrally a muscular ridge which divides the ventricle into two main cavities, a small left and a large right."

In ***Natrix piscator***, the muscular ridge (Fig. 7, B) is a relatively small structure, arising from the ventral wall of the ventricle towards the right of the middle line and projecting obliquely upwards from its base. Its dorsal half, curving downwards towards the right, bounds an extremely small ventro-lateral space (***cavum pulmonale***) from a larger cavity on its dorsal side and its anterior portion is attached to form the dorsal and dorsolateral wall of the pulmonary trunk (Fig. 7, C). The ***cavum dorsale*** is not divided into chambers but is continuous from its left end to its right. It has, however, two strong projections (Fig. 7 B), one on its roof and the other on its floor. These may be called the ***septoid processes*** ; they would press against each other during systole and thus prevent the blood on the left side from mixing with that on the right.

In this connection one might quote Rau (1924) who found that the "large right cavity" (***cavum dorsale***) in the ophidian ventricle is further

divided by a septum, the interventricular septum, into two cavities—the dorso-lateral right and the ventral one. The interventricular septum is particularly well developed. It is attached to the ventral wall of the ventricle and has a free dorsal border anteriorly which projects freely into the cavity of the ventricle. Posteriorly the septum is continued and separates the ventral cavity effectively from the others." As Rau gave a description of the muscular ridge in addition to this septum, it seems likely that the latter corresponds to the ***ventral septoid process*** described by me.

The auriculo-ventricular openings (Fig. 5) are situated more anteriorly than in *Varanus monitor* (*cf.* Mathur, 1944), and the auricles do not project into the lumen of the ventricle as ***intraventricular atria***. As the ventricle projects forwards on the left side, the left auricle leads directly into the definitive lumen of the ventricle at its posterior end, but the right auricle

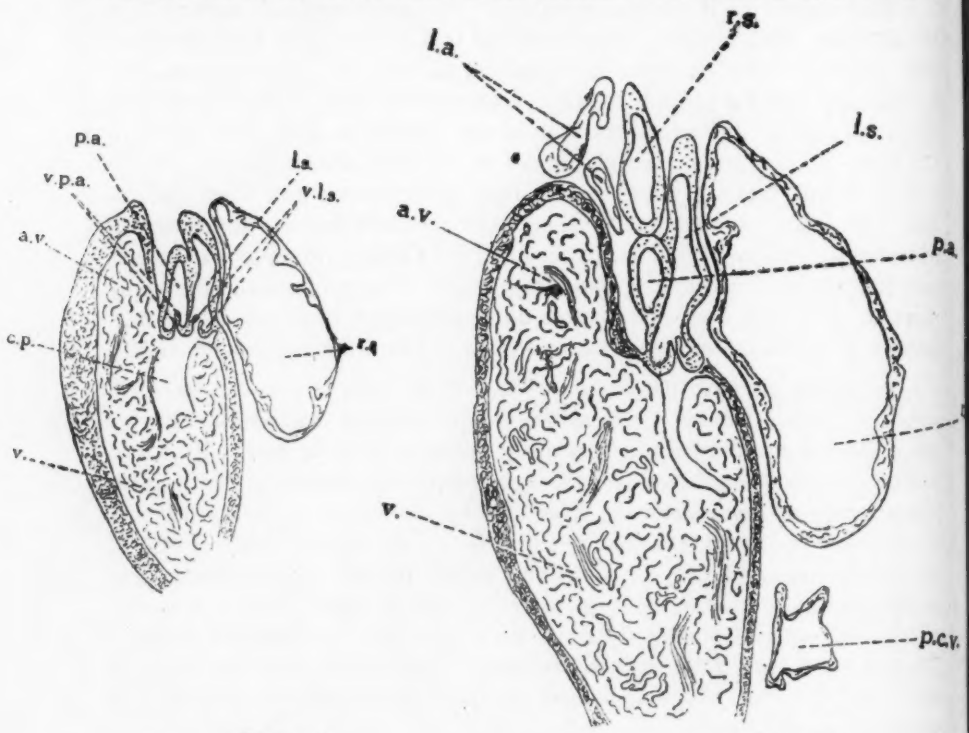

FIG. 8 (A) FIG. 8 (B)

FIG. 8. Horizontal longitudinal sections through the heart of *Natrix piscator* :—(A) at the level of the origion of the pulmonary and left systemic trunks; and (B) at the place of origin of the right systemic trunk. (×10). Abbreviations as in previous figures.

leads into a space, which has ventricular wall on its ventral and right sides and an atrial sort of covering on its dorsal aspect. It is only the left lobe of the right auricle which opens into the ventricle; the right lobe extends back behind this region as a structure quite separate from the ventricle.

Rau (1924) observed that in *Eunectes murinus* "the pulmonary artery arises ventrally from the cavum pulmonium (*sic.*); the left systemic artery springs as in Tiliqua at a short distance cranially from the origin of the pulmonary artery to the extreme right;" and "the right systemic arises even more cranially." I find a similar disposition in *Natrix piscator*. The openings of the pulmonary and left systemic arches appear to lie side by side almost at the same level in horizontal longitudinal sections (Fig. 8 A), but serial transverse sections leave no doubt that the latter (Fig. 7 C) is situated a little anteriorly to the former. The opening of the right systemic (Fig. 7 D and 8 B) as usual, lies immediately above that of the left systemic but is rather more anteriorly situated than the openings of the other two trunks. The pulmonary trunk takes its origin from the cavum pulmonale while the other two arise from the right part of the cavum dorsale. The origin of the systemic trunks almost from the same place where the right auricle leads into the lumen of the ventricle may be particularly noted.

III. The Heart of the Testudinata

The heart of testudinates, as first noted by Fritsch (1869) and later described by Hoffmann (1890) and Benninghoff (1932), corresponds in its external appearance to the general form of the body of these reptiles. It is broader[2] than long (the ratio of width to length being 3:2 according to Fritsch), is dorso-ventrally flattened, and has the apex of the ventricle almost completely rounded. Its situation, considerably behind the anterior border of the corselet, is regarded as a concomitant of its higher grade of organization (Hoffmann, 1890).

The displacement of the testudinate heart from the mid-longitudinal axis of the body is worth noting. In *Lissemys punctata* (Fig. 9) not only does the heart lie to one (right) side of the axis, but it is also tilted in such a way that its mid-longitudinal axis lies not parallel but at an acute angle to that of the body. Such a displacement and tilting, which is probably due to the retractility of the neck into the antero-mesial part of the trunk, leads to

[2] According to Rathke (1848), the heart in the embryos of *Chelone* is longer and narrower than in the adult, and according to Burne (1905) it was somewhat long and narrow in the specimen of *Dermochelys* (*Sphargis*) *coriacea*, studied by him. O'Donoghue (1918) pointed out that Burne's specimen was probably a young one.

considerable elongation of the left arteries and veins and a shortening of the right ones.

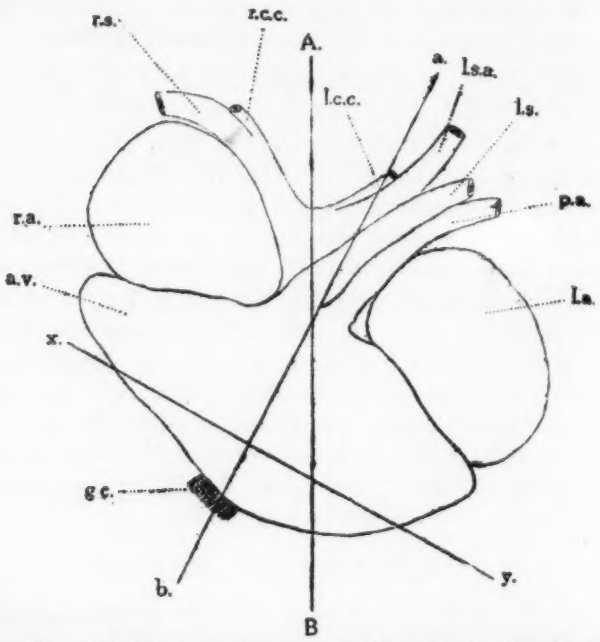

FIG. 9. The heart of *Lissemys punctata* (ventral view), showing the mid-longitudinal axis (AB) of the body, the mid-longitudinal axis (ab) of the heart as established by comparison with other reptiles, and the plane (xy) parallel to which transverse sections were cut.

g.c., gubernaculum cordis; *l.c.c.*, the left common carotid artery; *l.s.a.*, the left subclavian artery; *r.c.c.*, the right common carotid artery; other abbreviations as in previous figures.

As the general anatomy of the testudinate heart is well known (Rathke, 1848; Fritsch, 1869; Hoffmann, 1890; Burne, 1905; O'Donoghue, 1918; Hyman, 1922, etc.) we may deal here only with its minute anatomy. A careful study of the heart of ***Lissemys punctata*** (Bonnaterre) in serial sections (Fig. 10) shows that it resembles that of snakes to a great extent.

In the first place, the ventricle, like that of snakes, is seen in transverse sections to be produced forwards on the left side in front of the level of the auriculo-ventricular apertures. In superficial views, however, the extreme shortening of the heart in the antero-posterior direction almost completely conceals this left prolongation of the ventricle.

Secondly, the two auricles open almost at the same level close to the coronary sulcus. The left auricle opens into the left part of the ***cavum dorsale*** and the right auricle into the right.

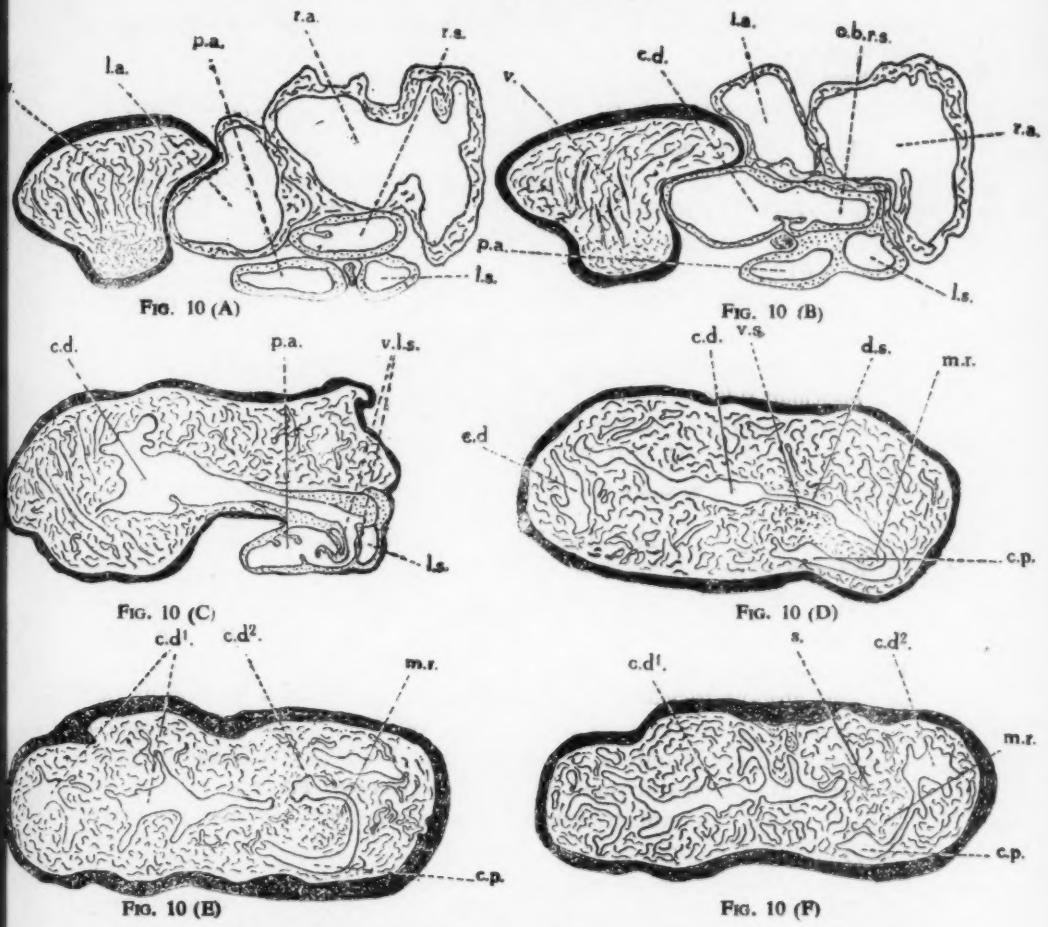

FIG. 10. Transverse sections through the heart of *Lissemys punctata*, section A being the most anterior and section F the most posterior (×8).

*c.d.*¹, the left portion, and *c.d.*², the right portion of cavum dorsale; *m.r.*, the muscular ridge; *o.b.r.s.*, the open base of the right systemic trunk; *s.*, the septum formed by the union of the dorsal and ventral septoid processes; *v.s.*, the ventral septoid process. (Other abbreviations as in previous figures.)

Thirdly, the pulmonary trunk arises behind the point of origin of the systemic trunks. The left systemic lies at its origin just beneath the right; and the two systemics receive their blood from the right part of the *cavum dorsale* immediately behind the auriculo-ventricular apertures. Comparison of successive transverse sections also establishes the fact that the left

anterior prolongation of the ventricle receives, as in snakes, arterial blood from the left auricle, while the venous blood from the right auricle passes backwards on the right side of the heart to the dorsal aspect of the muscular ridge and then across its free border into the *cavum pulmonale* below.

Fourthly, the dorsal and ventral *septoid processes* are present in the *cavum dorsale*. They, however, differ from those of snakes in two important features: first (Fig. 10 D), in correlation with the dorso-ventrally compressed nature of the cavum dorsale, they are very broad and flattened. Secondly (Fig. 10 E), in the posterior part of the ventricle they unite with each other to form a complete partition separating the left part (*cavum sinistrum*) of the *cavum dorsale* entirely from its right part (*cavum dextrum*), the latter alone being in communication with the *cacum pulmonale*. Posteriorly, the *muscular ridge*, which is considerably broad in the region bearing the free septoid processes, diminishes gradually in width, until close to the apical end of the ventricle (Fig. 10 F) it is discernible only as a small blunt growth on the septum, formed by the union of the two septoid processes.

IV. The Heart of the Loricata

(*a*) *General*

The essential features of the crocodilian heart are very well known. As pointed out by previous authors (Parker and Wiedersheim, 1907; Kingsley, 1926; Goodrich, 1930, etc.) the sinus venosus is subdivided by a septum; the large *atrio-ventricular* aperture is guarded by a muscular flap on its right (outer) side; the ventricle is completely divided by a *septum ventriculorum* into right and left chambers, the former containing venous blood alone and the latter arterial; and a small aperture, the *foramen Panizzæ*, puts the two systemic trunks into communication at their origin just distal to the valves.

(*b*) *Minute Anatomy*

The present account of the crocodilian heart, based on a thorough examination of successive transverse and horizontal sections of the heart of *Crocodilus palustris* Lesson, supplements our knowledge of this organ by a number of new important observations.

1. *Atrium Dextrum.*—The sinu-atrial aperture (Fig. 11) leads rather laterally into the dorso-lateral outer wall of the right auricle some distance behind the latter's anterior border. It is guarded by a pair of cranial and caudal valves, hanging freely into the lumen of the auricle. The *septum atriorum* is complete and unperforated. The right auricle is definitely larger than the left and its posterior part covers more than half of the right side of the ventricle. It leads downwards into the right chamber of the ventricle by

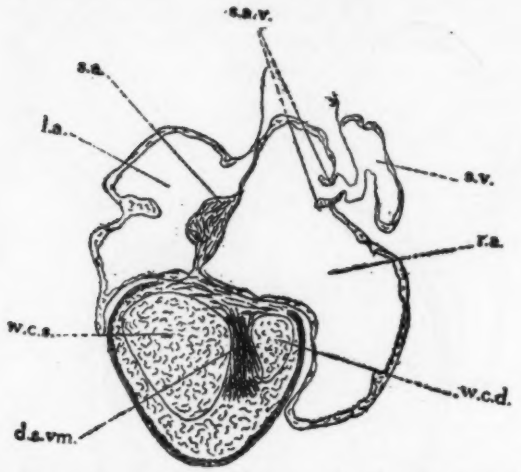

FIG. 11. Horizontal longitudinal section through the heart of *Crocodilus palustris*, showing the *sinu-atrial aperture* with its cranial and caudal valves. (×8).

d.s.vm., the dorsal attachment of the septum ventriculorum; *s.a.*, the septrum atriorum; *w.c.d.*, the wall of the cavum dextrum; *w.c.s.*, the wall of the cavum sinistrum. Other abbreviations as in previous figures.

the right *atrio-ventricular aperture*, which is situated on the dorsal wall of the ventricle close to the dorsal edge of the *septum ventriculorum*. The aperture is guarded by a pair of right and left valves (Fig. 12 A), attached by their dorsal and anterior edges, but having their posterior and ventral borders hanging freely into the lumen of the ventricle.

2. *Atrium Sinistrum.*—The opening of the pulmonary vein lies almost midway between the anterior border of the left auricle and the coronary sulcus. It is not guarded by any valves. The left auricle leads into the left chamber of the ventricle by the left *atrio-ventricular aperture* (Fig. 12 B) which is similar in its disposition and valves to the right one, but lies a little dorsal to the plane of the latter. The inner valves of both the atrio-ventricular apertures are developed on the terminal part of the *inter-auricular septum*.

3. *Ventriculus.*—The ventricle is completely divided into right and left chambers by a vertical partition, the *septum ventriculorum* (Fig. 13 A). The left chamber which we may call *cavum sinistrum* is almost half the size of the right (*cavum dextrum*) and some distance in front of the apical region, it receives blood from the left auricle by the left *atrio-ventricular aperture* (Fig. 13 B), which is provided by a pair of outer and inner valves. At the

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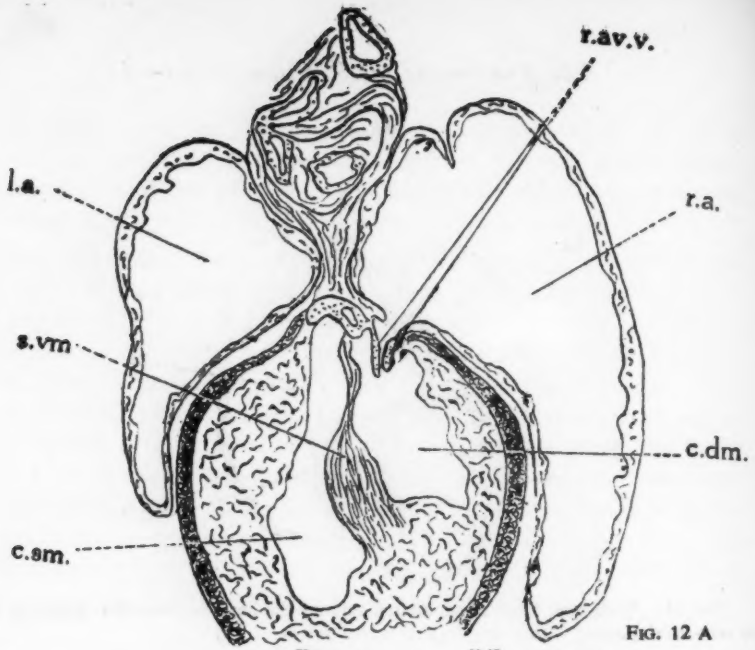

FIG. 12 A

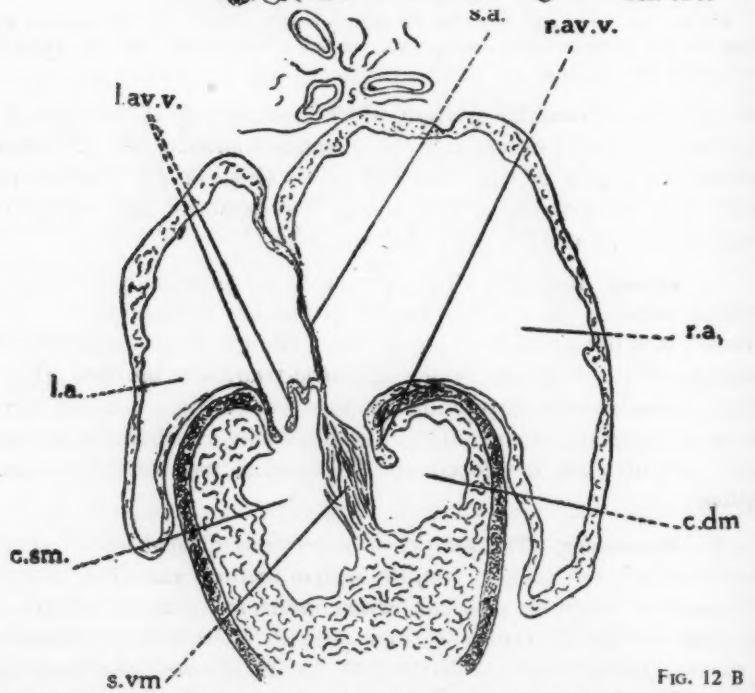

FIG. 12 B

FIG. 12. Horizontal longitudinal sections through the heart of *Crocodilus palustris*, showing the right (A) and left (B) atrio-ventricular apertures with their valves. (× 10).

c.dm., cavum dextrum ; ***c.sm.***, cavum sinistrum ; ***s.vm.***, the septum ventriculorum. Other abbreviations as in previous figures.

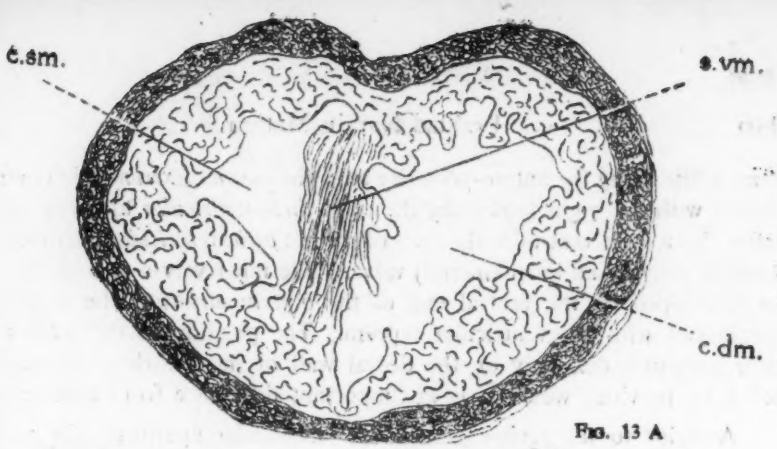

FIG. 13 A

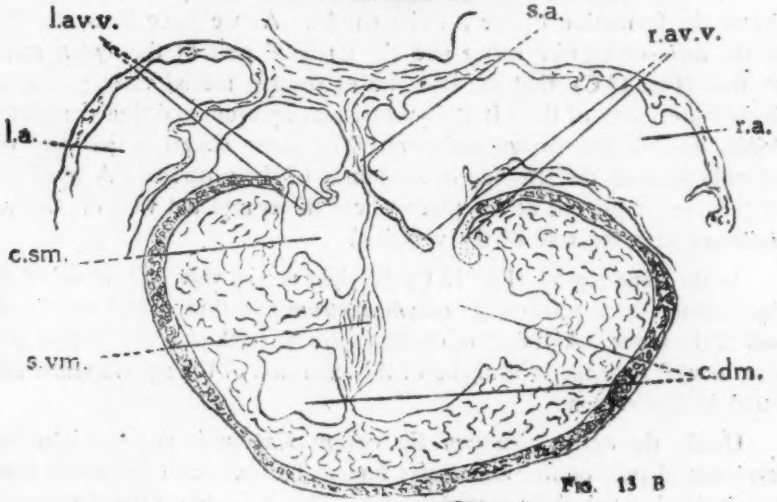

FIG. 13 B

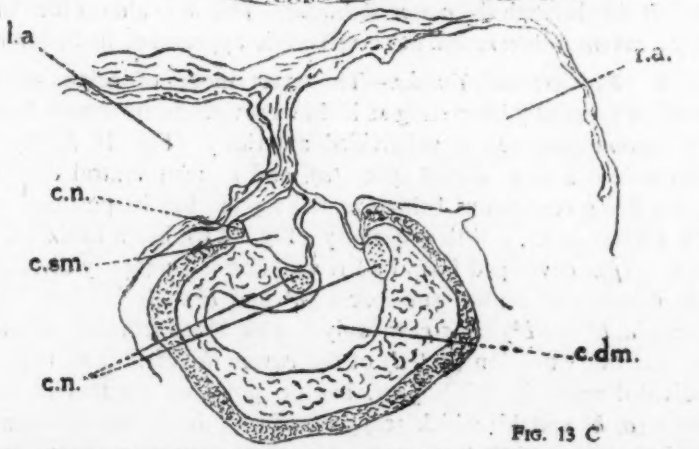

FIG. 13 C

FIG. 13. Transverse sections through the ventricle of *Crocodilus palustris*, showing (A) the disposition of the septum ventriculorum, (B) the atrio-ventricular apertures with their valves and (C) the formation of the arterial trunks. (×10).

c.n., the cartilaginous nodule. Other abbreviations as in previous figures.

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same plane along the antero-posterior axis, the *cavum dextrum* is in communication with the right auricle by the right *atrio-ventricular aperture*, but the latter lies a little ventral to the level of the left *atrio-ventricular aperture* and is guarded only by an inner (mesial) valve. The inner valves of both apertures are developed on the ventral end of the *septum atriorum*, the latter being continuous with the ventricular septum. The position of the *otrio-ventricular* apertures definitely on the dorsal wall of the ventricle, although not noted by previous workers, is an important difference from other reptiles.

Anterior to the region of the *atrio-ventricular* apertures, the ventricle shows the formation of the arterial trunks. As we trace forwards the fate of the *atrio-ventricular valves* and the terminal part of the *septum atriorum*, we find (Fig. 13 C) that the free end of the left mesial valve gets attached to the outer valve of the left atrio-ventricular aperture and thus completes the dorsal wall of the *cavum sinistrum*. The latter chamber lies here dorso-laterally towards the left side in the lumen of the ventricle. A small nodule of cartilage is present at the place where the completed wall of the *cavum sinistrum* joins the wall of the ventricle.

In the same region (Fig. 13 C) the right mesial valve gets attached to the right dorso-lateral wall of the *cavum dextrum* and thus completes the dorsal wall of the latter. A nodule of cartilage similar to the left one is also present here, as well as at the ventral end of the extension of the *septum ventriculorum* found in this region.

Unlike the *cavum sinistrum*, the *cavum dextrum* is not restricted to the dorso-lateral part of the ventricular lumen, but extends downwards towards the left side beneath the *cavum sinistrum*. Thus it is almost four times as large as the *cavum sinistrum* and has a crescentic appearance in transverse sections.

4. *The Arterial Trunks.*—The exact mode of origin of the arterial trunks is especially interesting as it shows several features not observed so far. The *cavum sinistrum* is subdivided anteriorly (Fig. 14 A) by an oblique septum into a large dorsal space (*a*), and a small ventral one (*b*), the two spaces being continuous behind as the septum has its posterior border free. The ventral space, a little anteriorly (Fig. 14 B), has a horizontal projecting process (*p*), developed from its left wall and incompletely dividing its lumen into dorsal and ventral sub-spaces (b^1 and b^2). It is clear that all these spaces (*a*, b^1 and b^2) can receive only aerated blood from the *cavum sinistrum*. The anterior extension ($c + d$) of the *cavum dextrum*, is seen to be a single undivided space in this region, has no point of communication with the spaces (*a*, b^1 and b^2) which really pertain to the *cavum sinistrum*. Further anteriorly (Fig. 14 C) the extension of the *cavum dextrum* is divided into a

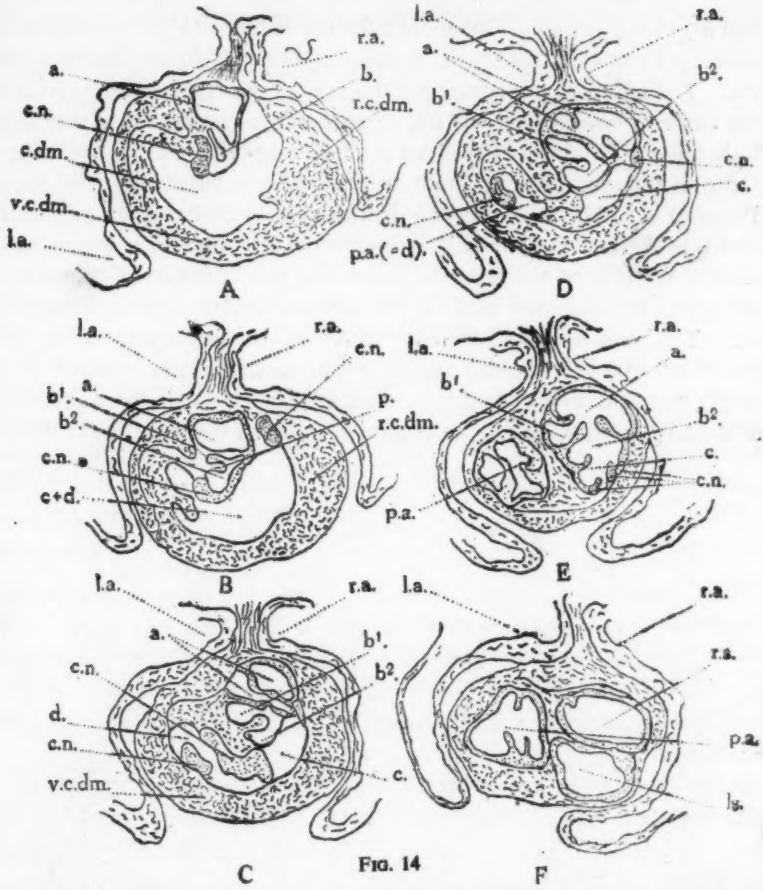

FIG. 14

FIG. 14. Transverse sections through the heart of *Crocodilus palustris* showing the mode of origin of the three arterial trunks. The section figured in A is the most posterior, that in F the most anterior. (×10).

r.c.dm., the right wall of the cavum dextrum; *v.c.dm.*, the ventral wall of the cavum dextrum. Other abbreviations as in previous figures.

dorso-lateral (*c*) and a ventro-lateral space (*d*), both spaces receiving non-aerated blood from the *cavum dextrum*. The ventro-lateral space (*d*) extends forwards as the *pulmonary trunk* and is distinctly cut off from the others in its forward course (Fig. 4, D, E and F). The spaces of the *cavum sinistrum* (*a*, b^1 and b^2), as well as the dorso-lateral space (*c*) of the *cavum dextrum*, however, show certain points of communication with each other before they form the systemic arches. The right systemic is formed by the spaces

(*a* and b^1) of the *cavum sinistrum*, but the ventral part (b^2) of its ventral space is excluded from this trunk. It, therefore, contains almost unmixed aerated blood. The left systemic comprises the ventral part (b^2) of the ventral space of the *cavum sinistrum* and the dorso-lateral space (*c*) of the *cavum dextrum* and, therefore, contains a mixture of aerated and non-aerated blood. According to previous authors, "a small aperture of communication (the foramen of Panizza) exists between the two aortic roots at their origin, just distal to the valves".[3] I have very carefully scrutinised successive transverse and horizontal sections of the heart of *Crocodilus palustris* Lesson, but have not found any communication between the arches distal to their valve-containing bases. The pulmonary trunk (Fig. 15 A) arises ventrally to the points of origin of the systemic trunks, and at the horizontal plane at which it takes its origin from the cavum dextrum, it completely separates the right systemic from the left, so that there is no communication of these arches at this level.

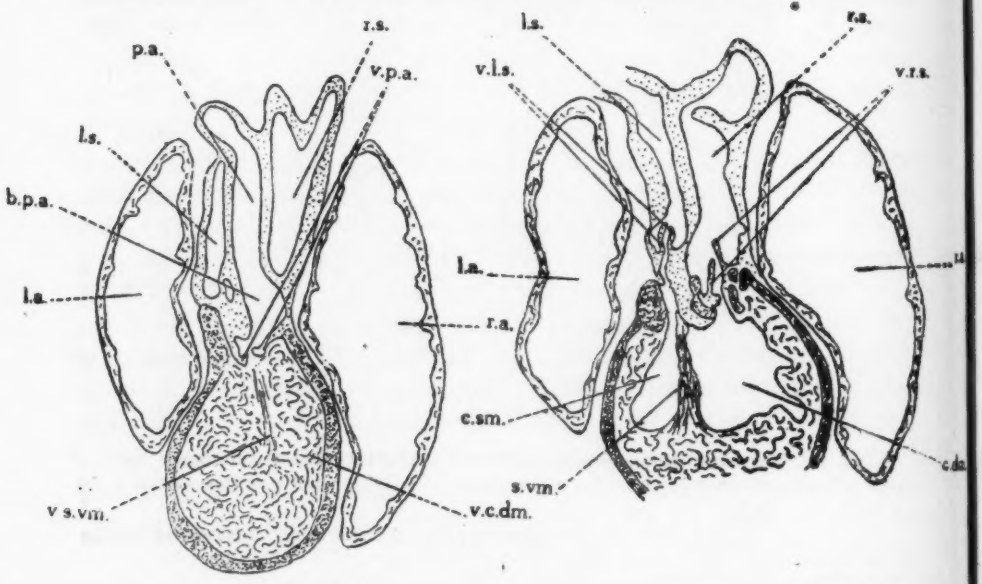

FIG. 15 A FIG. 15 B

FIG. 15. Horizontal longitudinal sections through the heart of *Crocodilus palustris*, showing the pulmonary trunk with its valves, and the absence of communication between the two systemic trunks. (×8).

b.p.a., the base of the pulmonary trunk ; ***v.r.s.***, valves of the right systemic trunk ; ***v.s.vm.***, the base of the septum ventriculorum ; other abbreviations as in previous figures.

[3] Quoted from Parker and Wiedersheim, 1907, p. 410.

A little dorsal to this plane the two systemics no doubt come to lie adjacent to each other, but the separating wall between them is devoid of any perforation (Fig. 15 B).

Although there is no communication in ***Crocodilus palustris*** between the systemic arches distal to their valves, as reported by Panizza and believed generally by comparative anatomists, there is, as shown by me in the account of the origin of the two trunks (Fig. 14) a small space (b^2), which, arising from the lumen of the cavum sinistrum becomes confluent with an extension of the cavum dextrum to form the left systemic. This space corresponds in my opinion to the communication between the bases of the right and the left systemic arches in *Varanus monitor* (*cf.* Mathur, 1944).

V. Summary

In the present paper, the author has studied the hearts of *Natrix piscator*, *Lissemys punctata* and *Crocodilus palustris* in the same way as he previously studied the heart of *Varanus monitor*. The more important features discovered by him are as follows:

(*a*) *Serpentes*

(1) The *sinu-atrial* aperture in snakes is not a transverse opening at right angles to the long axis of the heart, as generally believed, but is an elongated slit parallel to it. There is neither a *suspending ligament* nor a *sinu-atrial* channel as in *Varanus monitor*.

(2) The absence of valves at the opening of the pulmonary vein has been confirmed; Ray's suggestion about the rôle of the inter-atrial septum in prevention of regurgitation has been substantiated, and a subsidiary device for this purpose (dilatation of the terminal part of the pulmonary vein) is described for the first time.

(3) The auricles in snakes are not incorporated into the lumen of the ventricle and thus there are no *atria intraventriculare*.

(4) The ventricle is elongated and asymmetric, and its left side extends anterior to the level of the atrio-ventricular apertures as the ***anterior extension of the ventricle***. The coronary sulcus is oblique.

(5) In the *apical region* there is no *horizontal septum* and consequently no *cavum apicis dorsale* and *cavum apicis ventrale*, as in ***Varanus monitor***.

(6) The muscular ridge, though very similar in its general disposition to that of *Varanus monitor*, is smaller.

(7) The cavum pulmonale is an extremely small ventro-lateral space while the cavum dorsale is a single undivided cavity; the latter has two

strong projections, the *septoid processes*, developed one on its roof and the other on its floor. These processes, apparently, press against each other during systole and thereby prevent the blood on the left side from mixing with that on the right side.

(8) The *auriculo-ventricular apertures* and their valves in snakes are accurately described and their differences from those in the Sauria noted.

(9) The *pulmonary trunk* arises from the *cavum pulmonale*, and the left and right systemics from the right part of the *cavum dorsale*.

(*b*) *Testudinata*

(10) The asymmetrical disposition of the testudinate heart, due to the retractility of the neck, has been described and discussed.

(11) The sinu-atrial aperture is an oval and oblique slit, inclined towards the right side and opening into the terminal part of the right pre-caval vein.

(12) The two auricles open into the ventricle at almost the same level. The left auricle leads into the left part of the *cavum dorsale* and the right into its right part.

(13) There are no *atria intra-ventriculare* in turtles.

(14) An anterior extension of the ventricle is present on the left side as in snakes.

(15) In the *apical region* of the ventricle the *horizontal septum*, *cavum apicis dorsale* and *cavum apicis ventrale* are absent.

(16) As in snakes, *septoid processes* are developed in the *cavum dorsale* dividing the cavity into a left (*cavum sinistrum*) and a right one (*cavum dextrum*). It is noteworthy, however, that these processes in the Testudinata are fused together posteriorly to form a complete septum.

(*c*) *Loricata*

(17) The *sinu-atrial aperture* is situated on the outer dorso-lateral wall of the right auricle and has a pair of cranial and caudal valves.

(18) The *right atrio-ventricular aperture* is situated on the dorsal wall of the ventricle close to the dorsal edge of the septum ventriculorum and is guarded by a pair of right and left valves.

(19) The left *atrio-ventricular aperture* and its valves are similar to those of the right. This aperture, however, lies dorsal to the plane of the right.

(20) The opening of the pulmonary vein into the left auricle lies almost midway between the anterior border of the left auricle and the coronary sulcus.

(21) The ventricle is divided by a vertical partition (the ***septum ventriculorum***) into right and left chambers. These have been designated, in view of their homologues in other reptiles, as the ***cavum sinistrum*** and the ***cavum dextrum***.

(22) Anterior to the region of the atrio-ventricular apertures, the ventricle shows the formation of the arterial trunks. The free end of the left mesial valve gets attached to the outer valve of the left ***atrio-ventricular*** aperture and thus completes the dorasl wall of the ***cavum sinistrum***. Similarly the right mesial valve in the same region fuses with the dorso-lateral wall of the ***cavum dextrum*** and thus completes its dorsal wall. The two cavities, when traced forwards, give rise to the three arterial trunks.

(23) The exact origin of the pulmonary trunk has been established. It is found that the pulmonary trunk receives blood entirely from the ***cavum dextrum***. The left systemic receives it partially from the ***cavum sinistrum***, and the right systemic exclusive from the ***cavum sinistrum***.

(24) The ***Foramen Panizzæ***, generally believed to be of universal occurrence in the Crocodilia, is found to be absent in ***Crocodilus palustris***.

VI. Acknowledgments

My best thanks are due to Prof. Beni Charan Mahendra not only for his kind supervision and assistance, but also for translating the relevant German papers for me and for presenting me with a number of specimens from his personal collection.

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PROTEOLYTIC ENZYMES OF THERMOPHILIC BACTERIA—PART II

By N. N. Chopra, F.A.Sc.

Received November 19, 1945

Thermophilic bacteria are fairly widely spread and their proteolytic properties are called into play in several biochemical processes of importance in soil and agricultural chemistry and in the industry. These bacteria are capable of growing at unusually high temperatures. In Part I of this paper* attention has been drawn to the varied activities of these bacteria. A study of the proteolytic enzymes of these bacteria should prove useful. Besides the subject of microbial proteolytic enzymes has, as a whole, not been sufficiently investigated and further work with different types of micro-organisms and with better and more reliable experimental and analytical methods is necessary.

The present study is concerned with the proteinases of the three typical thermophilic bacteria—*Bacillus thermophilus*, *B. aerothermophilus* and *B. thermoacidurans*, cultures of which were obtained from the Lister Institute, London. Analytical and experimental methods here followed were, in general, similar to those described in Part I of this paper.

The endo- or exocellular nature of the proteinases of the thermophilic bacteria

A certain amount of controversy has centred round the question of the endocellular or exocellular nature of bacterial proteinases and in certain cases conflicting views have been expressed even with the same species of bacteria. Some confusion seems to have been caused by the failure to adequately distinguish between and separately determine the factors of multiplication, death of cells followed by autolysis and enzyme secretion by living cells. Virtanen and Tarnanen (1931) have stated that *Bacillus fluorescens* secretes a proteinase whose exocellular nature is assumed from the fact that the proteinase is found almost exclusively in the cell-free liquid. In a subsequent paper (1932) these authors stated that contrary to the general view on the subject, bacterial proteinases are exocellular in nature because filtrates from even young cultures of *Pseudomonas fluorescens* and *B. subtilis*, for instance, show proteolytic activity. Virtanen and Suolahti (1937) have

stated that contrary to Gorbach and Pirch (1936), proteinase of *B. fluorescens* liqu. is exocellular. Nevertheless Gorbach and Pirch (1937) have again observed that this proteinase is found in cultures only as a result of autolysis of dead cells. Maschmann (1938) has stated that *B. perfrigens* produces two proteinases, one of them being endocellular, and that the thiol activatable proteinase of the gas gangrene organism *Clostridium hystoliticum* is endocellular. On the other hand, Weil and Kocholaty (1937) and Kocholaty, Weil and Smith (1938) state that the thiol activatable proteinase of *Clostridium hystoliticum* is exocellular because the presence of the enzyme can be detected in filtrates from quite young cultures of this organism. Thus it would appear that conflicting conclusions have been reported even for the same species. It may be pointed out, however, that the mere fact that cultures are young is no guarantee that autolysis has not taken place.

In order to determine the endocellular or exocellular nature of the proteinases of thermophilic bacteria duplicate nutrient broth flasks were inoculated with each of the three bacteria and the proteinase content of the whole cultures, filtrates and washed cells was examined every six hours. The cells were obtained from the cultures by centrifuging at 3000 r.p.m. for thirty minutes and were re-suspended in distilled water so as to give the same volume as the amount of cultures they were reaped from. After thorough shaking 2 ml. were withdrawn for every estimation. Similarly 2 ml. of cell-free filtrates and 2 ml. of thoroughly shaken whole cultures were used for estimating the proteinase content of cell-free liquid and the total amount of proteinase in the culture respectively.

It is therefore obvious that the proteinase content of the whole cultures is always ahead of that of the filtrates. As the incubation time increases this difference becomes less and less. In the initial stages when the proteinase was just detectable in the whole cultures, the filtrates did not contain any and the cells accounted for all the proteolytic activity. Subsequently the difference between the proteolytic activity of the whole cultures and the filtrates roughly corresponded to the proteinase content of the cells. As the age of a culture increases the difference between the proteolytic activity of the whole culture and that of the cell-free filtrates becomes less and less and this progressive equalization corresponds to a decrease in the proteinase content of the cells. Thus the proteinase first appears in the cells and after some time it passes out into the filtrates. The decision whether the proteinases are really exocellular or endocellular is however dependent on the question whether when they diffuse out of the cell bodies into the surround-

TABLE I

Endocellular or Exocellular Nature of the Proteinases

Age of culture in hours	Whole culture		Cell-free filtrate		Suspension of cells	
	% Fall in initial Viscosity in 30 minutes	Increase in α-amino nitrogen, mgm./10 ml. in 48 hours	% Fall in initial viscosity in 30 minutes	Increase in α-amino nitrogen, mgm./10 ml. in 48 hours	% Fall in initial viscosity in 30 minutes	Increase in α-amino nitrogen, mgm./10 ml. in 48 hours
			B. thermophilus			
6 ..	0·00	0·00	0·00	0·00	0·00	0·00
12 ..	2·12	0·00	0·00	0·00	2·05	0·00
18 ..	8·30	2·37	0·00	0·00	7·80	1·89
24 ..	33·90	5·82	4·80	0·60	26·50	4·83
30 ..	57·80	10·53	29·15	2·13	28·55	7·81
36 ..	61·85	11·15	48·10	6·01	..	4·65
42 ..	60·05	11·01	50·35	9·00	10·15	2·11
47 ..	59·80	11·13	52·70	9·35	6·00	1·63
			B. aerothermophilus			
6 ..	0·00	0·00	0·00	0·00	0·00	0·00
12 ..	2·81	0·00	0·00	0·00	2·10	0·00
18 ..	6·65	1·12	0·05	0·00	6·50	1·09
24 ..	28·10	4·17	2·15	0·02	26·10	4·08
30 ..	49·50	8·73	23·50	3·13	26·35	5·91
36 ..	53·35	9·15	40·10	6·17	14·50	3·02
42 ..	56·00	9·97	53·70	7·83	3·20	2·11
			B. thermoacidurans			
6 ..	0·00	0·00	0·00	0·00	0·00	0·00
12 ..	0·00	0·00	0·00	0·00	0·05	0·00
18 ..	4·00	0·00	0·00	0·00	3·85	0·05
24 ..	29·95	4·37	12·05	1·15	17·30	3·37
30 ..	55·10	8·41	37·10	4·39	18·15	4·20
36 ..	55·60	9·17	43·50	6·16	..	3·15
42 ..	55·90	9·93	47·15	8·23	8·30	1·80
47 ..	54·05	9·83	48·10	8·25	6·05	1·45

ing medium the cells concerned are dead or alive. For ascertaining this point the course of proteinase increase or decrease in the whole cultures, in filtrates and in autolysed cells was re-determined and correlated with the estimation of total living and dead cells per ml. of the cultures.

Nutrient broth media was inoculated in each case from agar slant cultures and test samples were taken out aseptically after stated periods of incubation. After dilution smears were prepared, stained with carbol fuchsin and the total number of cells were counted. Simultaneously suitable decimal dilutions were plated on nutrient agar and counted after incubation for 48 hours. Estimation of total count by using the hæmocytometer, after rendering the diluted culture semisolid with agar so as to restrict bacterial movement, was found to be time consuming and inaccurate.

TABLE II

Hours after inoculation	Total No. of cells, 10^6/ml.	Viable cells, 10^3/ml.	Dead cells, 10^3/ml.	Whole culture		Filtrate		Cell suspension	
				% Fall in initial viscosity in 30 min.	Increase in α-amino nitrogen, mgm./10 ml. in 48 hours	% Fall in initial viscosity in 30 min.	Increase in α-amino nitrogen, mgm./10 ml. in 48 hours	% Fall in initial viscosity in 30 min.	Increase in α-amino nitrogen, mgm./10 ml. in 48 hours
				B. thermophilus					
4 ..	150	100	50	0·00	0·00	0·00	0·00	0·00	0·00
8 ..	..	..	..	0·00	0·00	0·00	0·00	0·00	0·00
12 ..	1,600	1,300	300	0·00	0·00	0·00	0·00	0·00	0·00
16 ..	10,500	7,000	3,500	1·25	0·00	0·35	0·00	0·80	0·00
20 ..	20,000	16,000	4,000	3·75	0·98	0·55	0·25	3·27	0·69
24 ..	30,000	15,000	15,000	42·70	6·72	24·10	3·97	20·15	3·19
				B. aerothermophilus					
4 ..	50	50	0	0·00	0·00	0·00	0·00	0·00	0·00
8 ..	700	..	..	0·00	0·00	0·00	0·00	0·00	0·00
12 ..	4,000	3,500	500	1·50	0·00	0·00	0·00	1·60	0·27
16 ..	15,000	10,000	5,000	3·70	0·78	0·30	0·15	3·55	0·65
20 ..	29,000	17,000	12 000	5·95	2·15	2·15	1·03	4·10	1·11
24 ..	32,000	17,000	15.000	39·40	7·32	20·05	4·05	20·90	3·79
				B. thermoacidurans					
4 ..	150	100	50	0·00	0·00	0·00	0·00	0·00	0·00
8 ..	600	350	250	0·05	0·00	0·00	0·00	0·00	0·00
12 ..	3.300	2,900	400	1·30	0·52	0·00	0·00	1·15	0·39
16 ..	29.000	23,000	6,000	4·85	2·25	0·75	0·10	4·15	2·07
20 ..	35,000	25,000	10,000	14·90	4·77	2·90	0·79	11·75	4·00
24 ..	39,000	18,000	21,000	38·75	8·15	19·80	3·07	22·80	4·98

An examination of the above data reveals that:—

1. death of the cells as determined by failure to multiply starts quite early after inoculation, in fact multiplication and death may be said to begin almost simultaneously; it is therefore inaccurate to say that in young cultures autolysis does not take place,

2. in case of the thermophilic bacteria little proteinase seems to be formed during the major part of the logarithmic phase of growth although the population of both dead and living cells is quite large,

3. cells reaped from thermophilic bacteria cultures which are yet in the logarithmic phase of growth also show little proteinase activity,

4. as soon as the logarithmic phase of growth is nearing completion in the cultures, proteinase can be detected in appreciable amount and then it increases rapidly both in the cells and in the surrounding liquid, the whole cultures still containing larger amounts than the cell-free filtrates and the proteinase contained in the cells themselves accounting for the difference and

5. the proteinase content of the whole cultures runs approximately parallel to the total cell count, whereas the proteinase content of the cell-free filtrates runs roughly proportional to the content of dead cells.

Unfortunately, these experiments had to be stopped after twenty-four hours, because after this the cells begin to show tendency towards formation of surface films which vitiate all cell counts. But it may be concluded that with the thermophiles the proteinase is generated within the cells after the maximum rate of multiplication has passed and that in all cases the proteinase is endocellular and appears into the surrounding liquid only after the cells have died.

Effect of calcium and magnesium on proteinase formation by the thermophilic bacteria and on the activity of the proteinase

Guillaumie (1935) has found that calcium activates trypsin. Merril and Clark (1928) found that in synthetic media certain bacteria produced a proteinase only in the presence of calcium and magnesium salts, although good growth was obtained even in their absence. Haines (1932) using *Pseudomonas* species and *Proteus* species has come to the conclusion that magnesium stimulates the growth of cells and calcium stimulates production of proteinase by the cells. These views on bacterial proteinases in general may have to be modified a little in view of the following findings with the thermophilic bacteria.

The procedure here adopted in studying the effect of a metal was to add a measured volume of the neutralised salt solution to the enzyme solution in such an amount that the desired final concentration would be obtained. The mixture was incubated at 30° C. for half an hour and its proteolytic activity was then measured in the usual way. Control experiments were performed in which the salt solution was replaced by an equal volume of distilled water.

TABLE III

Effect of Magnesium Salts—$MgSO_4 \cdot 7\ H_2O$

Source of Proteinase	Concentration of the salt in the enzyme solution	Initial rate, % Fall in initial viscosity in first 5 minutes	% Fall in initial viscosity in 30 minutes	Increase in α-amino nitrogen, mgm./10 ml. in 48 hours
B. thermophilus ..	None	7·38	50·60	6·97
	M/2000	7·42	..	7·49
	M/1000	7·73	52·15	7·85
	M/250	7·80	53·40	8·37
B. aerothermophilus ..	None	6·88	45·75	6·11
	M/2000	6·92	45·92	6·38
	M/1000	7·05	46·15	6·69
	M/250	6·90	45·80	7·31
B. thermoacidurans ..	None	7·13	48·35	6·71
	M/2000	7·21	49·00	6·95
	M/1000	7·37	49·30	7·31
	M/250	7·40	49·55	7·78

TABLE IV

Effect of Calcium Salts—$CaCl_2$

Source of Proteinase	Concentration of the salt in the enzyme solution	Initial rate, % Fall in initial viscosity in first 5 minutes	% Fall in initial viscosity in 30 minutes	Increase in α-amino nitrogen, mgm./10 ml. in 48 hours
B. thermophilus ..	None	7·28	50·05	6·91
	M/500	7·35	51·00	7·43
	M/250	7·35	51·30	7·95
B. aerothermophilus ..	None	6·85	45·15	6·05
	M/500	6·95	45·40	6·78
	M/250	6·96	45·90	7·17
B. thermoacidurans ..	None	7·05	47·60	6·59
	M/500	7·15	47·55	7·01
	M/250	7·20	47·90	7·48

The activating effect of both calcium and magnesium ions on the amount of α-amino acids liberated is moderate but definite and regular. The conclusion of Haines that presence of calcium salts increases the amount of proteinases produced may, however, be due simply to the conversion of the proteinase already present into a more active form. Haines did not compare the proteolytic acitivity of filtrates from cultures prepared in media containing calcium salts with filtrates from cultures prepared in ordinary media but after adding calcium salts a little while before testing for the proteolytic activity. With the thermophilic bacteria the proteolytic activity of filtrates obtained from cultures in broth containing calcium and magnesium salts was higher than that of filtrates obtained from cultures in plain broth but was nearly the same as that of plain broth culture filtrates which had been subsequently activated by the addition of calcium and magnesium salts. This is apparent from Table V. With Merril and Clark's synthetic medium it was found

TABLE V

A—Filtrates from plain broth cultures.
B—Filtrates from cultures made in broth containing calcium and magnesium salts.
C—Filtrates from plain broth cultures, but with calcium and magnesium salts added subsequently.

Source of Proteinase	Nature of filtrate	Initial rate, % Fall in initial viscosity in first 5 minutes	% Fall in initial viscosity in 30 minutes	Increase in α-amino nitrogen, mgm./10 ml. in 48 hours
B. thermophilus ..				
	A	7·35	50·30	5·67
	B	7·47	53·25	6·41
	C	7·42	53·90	6·37
B. aerothermophilus ..				
	A	6·83	47·35	5·01
	B	6·72	48·40	5·93
	C	6·87	48·75	5·90
B. thermoacidurans ..				
	A	6·31	50·80	5·63
	B	7·17	51·90	6·37
	C	7·11	51·95	6·38

that good growth could be obtained only when both calcium and magnesium salts were present, filtrates from such cultures had appreciable proteolytic activity although less than that of filtrates from broth cultures.

Effect of iron and copper on the proteinases of the thermophilic bacteria

Unlike papainases the proteinases of thermophilic bacteria are not suppressed by copper salts. The effect of ferrous and ferric salts is not marked except that at high concentrations of ferric ions a mild suppression of the activity of these proteinases is observed. The data about the effect of ferrous and ferric salts is, however, not reproduced here as the effect was inconsistent.

TABLE VI

Effect of Copper Salts —$CuSO_4 \cdot 5H_2O$

Source of Proteinase	Concentration of the salt in the enzyme solution	Initial rate % Fall in initial viscosity in first 5 minutes	% Fall in initial viscosity in 30 minutes	Increase in α-amino nitrogen, mgm./10 ml. in 48 hours
B. thermophilus ...	None	6·95	52·10	6·17
	M/1000	6·87	51·85	6·10
	M/250	7·05	52·35	6·28
	M/100	7·10	52·50	6·32
B. aerothermophilus ..	None	6·35	46·80	5·63
	M/1000	6·13	45·45	5·51
	M/250	6·29	46·75	5·59
B. thermoacidurans ..	None	5·80	40·50	4·98
	M/1000	5·95	41·30	5·02
	M/250	5·60	39·95	4·91
	M/100	6·05	42·95	5·10

These proteinases are therefore quite different from papainases in their response towards copper ions.

Effect of oxidising and reducing agents on the activity of the proteinases of thermophilic bacteria

It is quite well known that apart from pH, the activity of enzyme solutions is also affected by the presence of oxidising and reducing agents. In case of the proteinases, a study of the effect of oxidising and reducing agents throws some light on the nature and mode of action of the enzymes, hence the importance of this study. The effect of a number of reagents has been tried on the proteinases of *B. thermophilus*, *B. aerothermophilus* and *B. thermoacidurans* and the results are summarised in Tables VII, VIII and IX. Although the effect of oxidising agents is on the whole that of suppression of the proteolytic activity, yet the irregular nature of the data and the limited extent of the effect do not allow of any definite conclusions to be

reached. Agreement between duplicate sets of data was generally poor. Uniform and regular suppression of the bacterial proteolytic activity was obtained only with methylene blue and iodine. The latter prevents proteolytic activity in all cases.

The enzyme solution and the solution of the reagent were mixed in requisite proportions and incubated at 30° C. for half an hour before mixing with the substrate. For blank experiments an equal volume of distilled water was substituted for the solution of the reagent. The solutions of different reagents were pre-adjusted to the pH of the enzyme solutions.

TABLE VII

Effect of Oxidising and Reducing Agents

Ragent and concentration	*B. thermophilus*		
	Initial rate, % Fall in initial viscosity in 5 minutes	% Fall in initial viscosity in 30 minutes	Increase in α-amino nitrogen mgm./10 ml. in 48 hours
Nil ..	7·39	52·60	6·61
Potassium bromate M/100 ..	6·87	52·10	6·43
Potassium iodate M/100 ..	7·10	49·50	6·43
Methylene blue M/250 ..	6·27	43·10	5·53
Hydrogen peroxide M/100 ..	7·17	49·55	6·50
Potassium ferrocyanide M/200 ..	7·30	53·19	6·55
Potassium ferricyanide M/200 ..	7·11	50·00	6·47
Iodine M/350 ..	Nil	Nil	Nil

TABLE VIII

Effect of Oxidising and Reducing Agents

Ragent and concentration	*B. aerothermophilus*		
	Initial rate, % Fall in initial viscosity in 5 minutes	% Fall in initial viscosity in 30 minutes	Increase in α-amino nitrogen mgm./10 ml. in 48 hours
Nil ..	7·03	49·15	6·27
Potassium bromate M/100 ..	6·89	47·10	6·02
Potassium iodate M/100 ..	6·81	47·15	6·13
Methylene blue M/250 ..	5·97	42·00	5·43
Hydrogen peroxide M/100 ..	6·87	47·30	6·21
Potassium ferrocyanide M/200 ..	7·01	50·10	6·05
Potassium ferricyanide M/200 ..	6·83	46·90	6·21
Iodine M/350 ..	Nil	Nil	Nil

TABLE IX

Effect of Oxidising and Reducing Agents

Ragent and concentration	*B. thermoacidurans*		
	Initial rate, % Fall in initial viscosity in first 5 minutes	% Fall in initial viscosity in 30 minutes	Increase in α-amino nitrogen, mgm./10 ml. in 48 hours
Nil ..	6·95	49·90	6·07
Potassium bromate M/100 ..	6·87	49·00	6·02
Potassium iodate M/100 ..	6·89	48·15	6·11
Methylene blue M/250 ..	6·01	43·95	5·17
Hydrogen peroxide M/100 ..	6·87	48·05	6·00
Potassium ferrocyanide M/200 ..	6·97	51·10	6·05
Potassium ferricyanide M/200 ..	6·90	48·95	6·10
Iodine M/350 ..	Nil	Nil	Nil

Effect of peptone on the hydrolysis of gelatin by the proteinases of thermophilic bacteria

A concentrated solution of Witte's peptone was added to a 3% gelatin solution in McIlvaine's buffer at the optimum pH in such an amount that the final concentration of peptone would be 1%. Any alteration in pH incidental to the addition of peptone was adjusted. The addition of peptone increased the relative viscosity of the substrates by about 10%. But since fall of viscosity was being measured as per cent. of initial viscosity the error introduced was not considerable especially in view of the fact that the alteration in the rate of hydrolysis brought about by the addition of peptone was of a high magnitude. The enzyme solutions which were filtrates from not more than thirty-six hour old cultures showed little activity when tested against peptone alone. 2 ml. of the enzyme solution were used per 10 ml. of substrate.

TABLE X

Source of Proteinase	% Fall in initial viscosity in 30 minutes		Increase in α-amino nitrogen, mgm./10 ml.	
	Without Peptone	With peptone	Without Peptone	With Peptone
B. thermophilus ..	51·05	7·05	6·87	1·87
B. aerothermophilus ..	49·35	4·50	6·67	1·72
B. thermoacidurans ..	44·40	5·65	6·00	0·97

Thus peptone depressed the proteolytic activity of the bacterial proteinases.

Effect of peptone on proteolysis of gelatin substrates of different concentrations

It was now of interest to find out whether the effect of peptone is competitive or non-competitive with the substrate. Substrates containing 3·5, 2·7, 1·9 and 1·1% gelatin were mixed with Witte's peptone to give a final concentration of 1% peptone and any alteration in pH was adjusted. Duplicate sets were run without peptone.

TABLE XI

% Gelatin in substrate	% Fall in initial viscosity in 30 minutes		Increase in α-amino nitrogen, mgm./10 ml. in 48 hours	
	Without Peptone	With Peptone	Without Peptone	With Peptone
	Bacillus thermophilus			
3·5	40·10	20·35	6·11	3·47
2·7	40·00	10·50	5·15	2·03
1·9	48·15	7·10	4·02	0·95
1·1	52·23	3·00	3·83	0·35
	Bacillus aerothermophilus			
3·5	40·10	18·15	9·10	5·37
2·7	44·25	10·50	8·02	3·01
1·9	49·85	6·10	6·62	1·69
	Bacillus thermoacidurans			
3·5	43·15	22·10	7·38	4·25
2·7	46·65	8·05	6·32	2·91
1·9	48·10	4·90	5·43	1·70
1·1	51·30	2·10	4·10	0·95

It is therefore obvious that in every case the extent of depression in proteolytic activity caused by the presence of peptone decreases with increase of gelatin concentration in the substrate, *i.e.*, peptone depression is competitive with the substrate. It appears that although peptone itself is not hydrolysed by the enzyme which hydrolyses gelatin, yet it competes with the gelatin for attachment to the active group in the enzyme molecule, or at a group which is so near the active group of the enzyme molecule that through spatial hindrance the peptone molecule can prevent the attachment of the gelatin to the enzyme.

SUMMARY

1. The thermophilic bacteria *Bacillus thermophilus*, *B. aerothermophilus* and *B. thermoacidurans* produce very active proteinases which appear to be

endocellular in character and are found in culture filtrates only as a result of autolysis of the cells.

2. Filtrates obtained from broth cultures containing calcium and magnesium salts are proteolytically more powerful than those obtained from cultures prepared with ordinary broth, but the effect of these metals is on the activity of the proteinases and not on the quantity of the enzyme produced.

3. Unlike papainases the proteinases of the thermophilic bacteria are not affected by copper ions.

4. Activity of the proteinases of thermophilic bacteria is suppressed by methylene blue and is prevented by iodine.

5. Although peptone itself is not hydrolysed by the proteinases of thermophilic bacteria, yet it hinders the hydrolysis of gelatin. The evidence obtained tends to show that the effect of peptone is probably due to competition by peptone with the gelatin for attachment to the active centre of the enzyme molecule, or a centre very close to the active centre.

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THE EFFECTS OF PHOSPHATE DEFICIENCY ON RICE

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Introduction

From the standpoint of agronomy phosphoric acid reputedly affects the root system, tillering and seed production in crops. A deficiency of phosphoric acid causes stunting of the root, stem, and leaves, and depresses tillering, the number of productive tillers, and grain formation. As regards the composition of the crop, phosphoric acid deficiency leads to a low phosphorus content in the vegetative portion, and to an accumulation of amides and amino-acids in the plant. The absorption of potassium by the plant seems to be also depressed by this deficiency.

Phosphate manuring, if applied without undue delay, overcomes the effects of phosphate deficiency and increases growth, yield and phosphoric acid content of the crop. The latter result is of particular significance in the feeding of animals in areas of phosphate deficiency.

Although the importance of phosphoric acid in the manuring of rice is generally recognised no systematic attempt appears to have been made to demonstrate the effects of phosphoric acid deficiency on this crop.

The earliest scientific work on the phosphate manuring of rice was apparently undertaken by Kellner (1890). He used an acid soil of volcanic origin in Japan which appeared to be rich in total phosphoric acid and nitrogen as judged by soil analysis. The ultimate object of his work was to deduce the optimum rates of application of the manures required by the rice crop. There was a high response to phosphate and a moderate response to nitrogen

but no response to potash. Kellner's work was comprehensive as he studied the effect of various doses of N, P and K and analysed the crop.

In 1916 Warth reported the results of some preliminary experiments with the soils of certain experimental farms of Burma. He found that the figures for available phosphoric acid (Dyer) were unreliable as they did not correspond with the response obtained to phosphate manuring. In an extension of this work Warth and Po Shin (1919) identified certain areas of phosphate deficiency in Lower Burma and carried out pot culture experiments with several soils from that region. They found that response to phosphate manuring was more in accordance with the total phosphoric acid content of the soil than with the 'available' phosphoric acid content found by Dyer's method. The response was however complicated by simultaneous nitrogen deficiency. In fact, very few of the soils examined by them gave a response to phosphoric acid alone. Crop analysis was confined to the determination of phosphoric acid in straw.

Carberry (1933) carried out experiments on rice in sand culture to study the effects of a deficiency of each of the nutrients, N, P, K, Ca and Mg. Omission of P or K was reflected in the composition of the straw but not of the grain. In the absence of sufficient supplies of N and P the plants were often very high in silica content. No observation was recorded on the colour or morphological features of plants subjected to the deficiencies.

Sircar and Sen (1941) studied the nitrogen metabolism in the leaves of rice grown in sand culture under different degrees of phosphate deficiency. The phosphorus content of the leaves was not determined but with reference to the external concentration of this nutrient it was found that the total and the protein nitrogen decreased with decrease of phosphate supply. On the other hand, the amide-N and the amino-acid-N increased with decreasing phosphate supply. Further it was found that ear formation was inversely related to the phosphate supply. The plants receiving the highest dose of phosphoric acid produced no earheads, those receiving ⅓rd of the full dose produced earheads in 40 per cent. of them, while all the plants produced earheads when the phosphoric acid was reduced to 1/27th of the full dose.

In soil surveys conducted in India it has been found that phosphate deficiency is fairly widespread in the rice growing areas of Madras (Ramiah, 1937), Bihar (Sethi, 1943) and elsewhere. The response to phosphate manuring is however poorly correlated with soil analysis.

The existence of phosphate deficiency in the rice areas of U.S.A., British Guiana, and Malaya and poor response to phosphate manuring have been

reported in the literature. Thus in U.S.A., Kapp (1936) found no response to phosphate though the soil used by him was very low in available phosphoric acid. In British Guiana, Duthie (1940) found that numerous samples of rice soils showed a complete lack of available phosphoric acid (Truog's method) and that despite this deficiency the yields of rice were depressed by applications of superphosphate at 1 to 50 cwts. per acre. Again, in experiments carried out in Perak in North Malaya by the Imperial Chemical Industries (Malaya) Ltd.,* it was found that manuring with Nicifos caused a striking improvement in the growth of rice in the early stages on the phosphate deficient soils of the area and that the initial improvement disappeared gradually during the later stages of growth so that at harvest there was little difference between manured and unmanured plots. In this case it was suggested that an unknown limiting factor was operative and that the large variations in pH of these soils observed by Dennett (1932) might have an important bearing on the problem. It was further observed that in control plots the seedlings suffered from a disease known as " Penakit Merah " (red disease) believed to be caused by the deposition of iron compounds round the roots of the plants and that seedlings manured with Nicifos were not affected.

Gile and Carrero (1915) observed that the ash of the roots of upland rice contained up to 8·48 per cent. of ferric oxide. They assumed that this high figure for iron was due to contamination from the soil. The crop had been liberally fertilized with N, P and K but the soil was apparently calcareous.

According to Ramiah (1937), phosphate manuring has very little effect on the root development of rice.

Gericke (1930) found in water culture that the rice plant can make full growth without further addition of phosphoric acid provided it had received this nutrient for an initial period of about six weeks.

On the whole, it appears that the effects of phosphate deficiency on the rice plant have not been fully understood. When the facts are fully appreciated it may be possible to base manuring practice on plant symptoms instead of on trial and error methods of manuring or on the relatively unreliable data for available phosphoric acid contents of soils by empirical methods of analysis.

In connection with investigations on the nutritional disorders of the rice plant carried out in Burma large areas were found to be deficient in phosphoric acid but in most cases the deficiency was complicated by simultaneous

* The information regarding work in Malaya contained in this paragraph was obtained hrough the courtesy of the I.C.I. (Malaya), Ltd.

deficiency of other nutrients. It was however found that the soil of Kelingon Seed Farm at Yedashe, Central Burma, produced low yields of rice mainly due to phosphate deficiency. In the present article it is intended to describe the results of experiments carried out in Kelingon soil to determine the effects of phosphate deficiency on rice and to overcome these effects by manuring.

Methods and Materials

Standard methods were used in analysis (A.O.A.C. 1937; Wright, 1937, 1938).

Plant-growing experiments were carried out with the selected soil in pots and in the field. The symptoms of phosphorus deficiency were confirmed by the solution culture method.

The soil sample used in the present work was taken from the fields at Kelingon Seed Farm, near Yedashe in Central Burma. The soil is typical of a large area in Toungoo subdivision. The annual rainfall in the area is about 80 inches. The seed farm was cleared from grazing ground and opened in 1933. In spite of the virgin condition of the soil the annual yield per acre for the three years, 1935–38, was only 17·5 baskets (about 750 lbs.) of paddy grain. Experiments to improve the yield were instituted in 1938–39.

The soil sample used in this work was a heavy loam tending to be clayey in the subsoil. This soil has developed from Irrawadian sediments belonging to the Tertiary group which is distributed very extensively in Burma. It is very ferruginous, usually sandy, and carries fossil wood as a characteristic constituent (Chhibber, 1935).

Inspection of the profile showed no important horizon differentiation except for the surface layer which was somewhat darker than the subsoil. The colour was reddish pink throughout. There was no darkening in the subsoil as is usual in waterlogged rice soils. For laboratory examination, samples were taken at convenient depths down to 4 ft. The analytical data are shown in Table I.

The data of Table I show that the Kelingon soil is poor in several plant nutrients as judged by ordinary standards. Nitrogen appears to be present in satisfactory quantity. As regards phosphoric acid this is one of the poorest soils so far examined in Burma. The percentages of total and available phosphoric acid are extremely low. The existence of phosphate deficiency was confirmed by the tomato test (Mayer, 1930). The soil reaction is not too acid as rice is known to tolerate even higher acidity (Aso, 1930; Doyne and Glanville, 1932). The low base status and the high lime requirement

TABLE I

Analysis of Kelingon soil, Yedashe, Burma

Items	0–6"	6"–1'	1'–2'	2'–3'	3'–4'
CaO per cent. by con. HCl extraction ..	0·0280	0·0227	0·0216	0·0218	0·0224
MgO ,, ,, ,, ..	0·1120	0·1040	0·1360	0·1400	0·1760
K_2O ,, ,, ,, ..	0·0448	0·0755	0·0838	0·0996	0·1180
Total P_2O_5 ,, ,, ..	0·0149	0·0366	0·0383	0·0373	0·169
Available P_2O_5 per cent. by Dyer's method	0·0015	0·0015	0·0016	0·0014	0·0014
Organic carbon per cent. ..	0·81	0·54	0·30	0·24	0·21
Organic nitrogen ,, ..	0·0826	0·0560	0·0434	0·0322	0·0280
pH in water ..	5·04	5·08	5·00	4·93	4·93
pH in M. KCl ..	3·86	3·68	3·58	3·65	3·60
Lime requirement as per cent. of $CaCO_3$ (Hardy and Lewis)	0·276	0·434	0·478	0·511	0·493
Exchangeable bases millieqts. per cent..	2·04	2·34	2·14	2·00	2·44
Coarse sand per cent. ..	9·0	8·0	6·4	11·0	11·5
Fine sand ,, ..	31·8	22·7	18·4	17·4	19·5
Silt ,, ..	38·1	33·3	30·5	26·5	25·7
Clay ,, ..	20·4	34·5	42·2	42·8	42·5
Moisture lost at 100° C. per cent. ..	1·31	2·05	2·39	2·43	2·34

suggest that the soil may respond to treatment with bonemeal and other insoluble phosphates.

Before the analysis of the soil was carried out it had bee suggested that the Kelingon soil required the addition of green manure, farmyard manure or other forms of organic matter. The carbon and nitrogen contents of the soil did not agree with the suggestion and actual experiments with organic additions gave disappointing results.

On the whole it appeared probable that the main cause of the low productivity was a deficiency of phosphoric acid. The experiments described in this article confirmed that view and the opportunity was therefore taken to study the effects of phosphoric acid deficiency on the morphological characters, yield and chemical composition of the rice plant.

EXPERIMENTAL DATA

(*a*) *Pot culture experiments to determine the nature of the deficiency in the soil.*—Soil from an unmanured field was collected to a depth of 6 inches. It was air-dried, crushed and after passing through a sieve of ¼th of an inch mesh was filled into pots holding 22 pounds. Sufficient space was left in each pot to hold water to a depth of 6 inches. The soil was watered early in June and thoroughly puddled within a week. Thereafter the soil was left covered with water until required for transplanting. Distilled water was used throughout the experiment.

In one or two pots prepared as above seedlings were raised from Taungdeikpan seed which was sown on 21st June 1940. Uniform seedlings were uprooted and transplanted on 3rd August 1940 into pots kept ready for this purpose. Each pot received one seedling. No water was added for five days after transplanting so as to allow the seedlings to get established. Watering was then continued. After another week the pots were treated with manures applied as solution. The treatment for each pot was reckoned on the basis of 120,000 pots per acre. The treatments were at the rate of 80 lbs. per acre of N, P_2O_5 or K_2O in the form of ammonium sulphate, pure orthophosphoric acid, and potassium sulphate respectively. There were eight treatments in all, namely, O, N, P, K, NK, PK, NP, and NPK. Each treatment was replicated four times.

When the plants had grown sufficiently the depth of water was maintained throughout at about 6 inches. No drainage was provided. After the earheads had developed fully the water was allowed to dry out so as to permit the plant to ripen satisfactorily. The straw and earheads were harvested separately on 16th December 1940 and air-dried. The grain was threshed and winnowed and then weighed. The chaff was weighed with the straw.

During the growth of crop, observations were made on the plants in each pot so as to discover the nature of the soil deficiency and its effects on the plant characters. Inspection of Plates V (*a*) and V (*b*) shows that the soil responds only to phosphoric acid. The plant measurements are shown in Table II.

TABLE II

Characteristics of rice plants grown in Kelingon soil

Treatment	Observations on 14-9-40—Mean Values				Mean tillers per pot	
	Height of plant cm.	Length of leaf cm.	Maximum width of leaf mm.	Index of leaf area sq. cm.	14 9-40	14-10-40
O ..	57	32·1	3·3	10·9	0	4
N ..	61	30·7	3·8	12·5	0	4
P ..	71	45·2	6·8	30·7	4	10
K ..	62	33·7	4·1	14·9	0	4
NK ..	63	34·8	4·3	17·2	0	3
PK ..	87	57·0	9·3	53·3	5	12
NP ..	84	49·9	9·8	48·9	4	13
NPK ..	91	59·0	11·9	66·1	7	16

The data of Table II show that the plants treated with phosphoric acid were taller and had leaves with greater length, width and area than plants which

had received no phosphoric acid. It must be pointed out however that when measurements were taken in October these contrasts were not so prominent. Obviously, therefore, it is desirable to take the morphological observations as early as possible. As regards tillering the plants treated with phosphoric acid started producing tillers at an early date in contrast to the plants without phosphoric acid, and the contrast in tiller number was maintained throughout the life of the plants. The morphological characters are shown in Fig. 1 which brings out clearly the effect of treatment on the different characters and the close correlation between these characters. The large differences in the morphological characters are reflected in the yield of crop and the high correlation between these two groups of data is brought out in Fig. 2.

Some very remarkable contrasts were observed in the colour of the plants as may be seen from Table III.

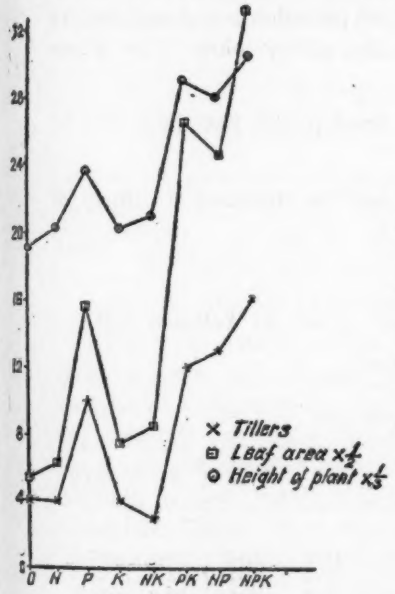

Fig. 1

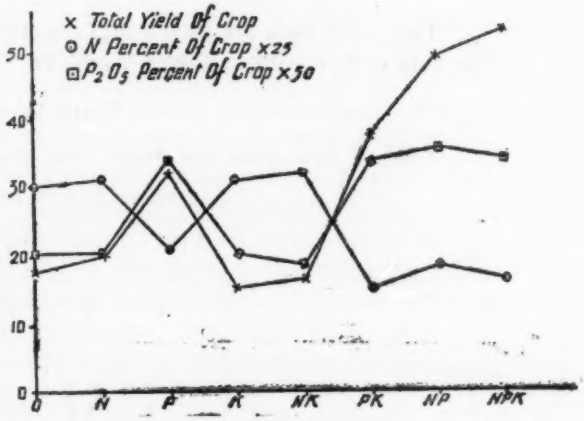

Fig. 2

Fig. 1. Response of rice to different treatments in Kelingon soil.
Fig. 2. Relation of yield and composition of rice to phosphate treatment in Kelingon soil.

In the absence of phosphoric acid the plants had a bluish green colour whereas those treated with phosphoric acid had a pale green or yellowish green colour. This difference in colour persisted until the date of harvest in December. When observed in November the phosphate-treated plants

TABLE III

Colour of rice plants receiving different treatments in Kelingon soil

Treatment	Remark on colour of plants on 14-10-1940
O ..	Bluish green—No leaf drying or yellow colour
N ..	Bluish green—Outer leaves drying without turning yellow
P ..	Pale green—Outer leaves showed yellow colour on drying
K ..	Dark bluish green—Leaves had brown spots but no yellow colour
NK ..	Dark green—Outer leaves turning yellow
PK ..	Pale yellowish green—Outer leaves turning yellow
NP ..	Pale green—Spotting and brown patches on leaves
NPK ..	Pale yellowish green—Outer leaves yellowing

showed much drying of the older leaves which had turned yellow whereas in all other cases leaf drying was quite small and there was no yellow colour. Even at harvest on 16-12-1940 the plants without phosphoric acid were mostly green and unripe in contrast with the ripe golden yellow colour of the phosphate treated plants.

Observations were also made on root development but these will be presented at a later stage.

The yield data are shown in Table IV and the statistical treatment of the data is dealt with in Tables V and VI.

TABLE IV

Yield of rice straw and grain from plants grown in Kelingon soil

(Data are in grams per pot)

Replication	Treatments							
	O	N	P	K	NK	PK	NP	NPK
Straw—								
A ..	7·1	10·3	20·3	7·5	11·8	21·7	24·9	27·2
B ..	10·0	9·2	16·9	5·2	7·6	25·4	28·3	34·7
C ..	9·6	11·4	18·5	7·8	8·2	18·6	19·9	32·6
D ..	8·9	9·1	19·6	9·9	10·4	18·3	24·5	32·7
Total straw ..	35·6	40·0	75·3	30·4	38·0	84·0	97·6	127·2
Mean straw ..	8·9	10·0	18·8	7·6	9·5	21·0	24·4	31·8
Grain—								
A ..	7·7	10·7	15·2	8·9	10·5	15·9	26·3	26·6
B ..	11·5	9·3	16·1	4·8	5·1	15·9	27·3	25·4
C ..	8·2	10·7	16·5	7·9	5·5	16·3	22·5	24·8
D ..	7·5	9·0	16·2	9·3	6·6	18·8	24·6	26·0
Total grain ..	34·9	39·7	64·0	30·9	27·7	66·9	100·7	102·8
Mean grain ..	8·7	9·9	16·0	7·7	6·9	16·7	25·2	25·7

TABLE V

Analysis of variance of yield of grain shown in Table IV

Sources of Variation	Degrees of freedom	Sum of squares	Mean variance	Calculated values of t	Level of significance
Replication ..	3	5·97			
Treatments—N ..	1	172·05	172·05	7·79	P = 0·001
P ..	1	1265·05	1265·05	21·2	P = 0·001
K ..	1	3·78	3·78		
NK ..	1	2·42	2·42		
PK ..	1	13·78	13·78	2·20	P = 0·05
NP ..	1	157·53	157·53	7·45	P = 0·001
NPK ..	1	1·62	1·62		
Residual error ..	21	59·56	2·836		
Total ..	31	1681·76			

Standard error of each total yield = ± 3·368.

Critical difference between two total yields for significance at 1 per cent. level = 13·48.

TABLE VI

Interaction of N and P (Yield of grain)

	No Nitrogen	With Nitrogen	Total
No phosphate ..	65·8	67·4	133·2
With phosphate ..	130·9	203·6	334·5
Totals ..	196·7	271·0	

Critical difference between two totals for significance at 1 per cent. level = 26·96.

The yield data show the remarkable effect of phosphoric acid. Very large increases are obtained in the presence of this nutrient. From the analysis of variance it may be seen that the main effect of phosphoric acid has absorbed practically three-fourths of the total sum of squares and hence the dominant effect of phosphoric acid is confirmed. The main effect of nitrogen is also highly significant. Among the interactions, N × P is highly significant. Analysis of straw yields led practically to the same conclusions as in the case of grain.

The composition of the mature crop is shown in Table VII. The interrelationships between plant composition, yield and certain plant characters were determined by calculating the appropriate coefficients of correlation and regression which are shown in Table VIII.

The data of Table VII reveal two very noteworthy features. When the treatment did not include phosphoric acid both straw and grain contained abnormally low percentages of phosphoric acid but when this nutrient

TABLE VII

Composition of rice plants grown in Kelingon soil in pots

(Percentages on oven-dry materials)

Treatment	Straw							Grain	
	N	P_2O_5	K_2O	CaO	MgO	Fe_2O_3	Mn	N	P_2O_5
O ..	0·77	0·046	0·98	0·29	0·096	0·136	0·040	1·62	0·365
N	0·81	0·038	0·92	0·28	0·088	0·136	0·040	1·70	0·355
P ..	0·64	0·084	1·20	0·31	0·104	0·204	0·031	1·03	0·636
K ..	0·82	0·048	1·22	0·37	0·088	0·136	0·040	1·74	0·345
NK ..	0·94	0·038	1·25	0·36	0·096	0·136	0·066	1·76	0·305
PK ..	0·32	0·106	1·50	0·33	0·160	0·136	0·048	0·92	0·626
NP ..	0·39	0·094	1·30	0·38	0·168	0·136	0·062	1·09	0·613
NPK ..	0·35	0·088	1·51	0·35	0·176	0·136	0·057	0·99	0·618

TABLE VIII

Relationship between composition, yield and plant characters

Related quantities x y	Correlation coefficient	Calculated value of t	Value of t for significance at 1 per cent. level	Regression coefficient b_{xy}
Grain yield and tillers ..	0·9419 ± 0·1270	7·42	3·36	1·2468 ± 0·2860
,, mean leaf area	0·9018 ± 0·1764	5·11	,,	0·1208 ± 0·0236
,, and N per cent. in grain	0·8876 ± 0·1881	4·72	,,	67·67 ± 14·33
,, and P_2O_5 per cent. in grain	0·8825 ± 0·1920	4·60	,,	168·17 ± 36·57
N in grain and P_2O_5 in grain ..	0·9893 ± 0·0596	16·60	,,	2·478 ± 0·1483
N in grain and mean leaf area	0·9059 ± 0·1729	5·24	,,	0·00159 ± 0·00030
P_2O_5 in grain and mean leaf area	0·8479 ± 0·2170	3·90	,,	0·00595 ± 0·00152
Mean leaf area and P_2O_5 in straw	0·9064 ± 0·1707	5·31	,,	694·18 ± 133·80

was supplied the crop absorbed it in increasing amounts and the maximum percentage found in the grain was equal to the normal value. The maximum percentage found in the straw was however far below the normal phosphoric acid content of well manured straw produced in fertile soils. The second feature is the inverse relation between phosphate manuring and the nitrogen content of the crop. When the treatment did not include phosphoric acid both the straw and the grain contained abnormally high percentages of nitrogen, but in the crop manured with phosphoric acid the nitrogen content was normal though the straw treated with pure orthophosphoric acid was

fairly high in itrogen. The normal values found in the variety Taungdeikpan are about 0·4 per cent. and 1·0 per cent. of nitrogen and 0·2 per cent. and 0·6 per cent. of phosphoric acid for straw and grain respectively. Nitrogen figures much higher and phosphoric acid figures much lower than these limits are definite indications of nutrient deficiency when the Taungdeikpan variety of rice is used as the indicator plant.

The correlation between the phosphoric acid content and the nitrogen content of the crop was found to be negative and highly significant (Table IX, Fig. 3). The yield of grain was inversely correlated with the nitrogen content and directly with the phosphoric acid content (Fig. 4). In fact, the corre-

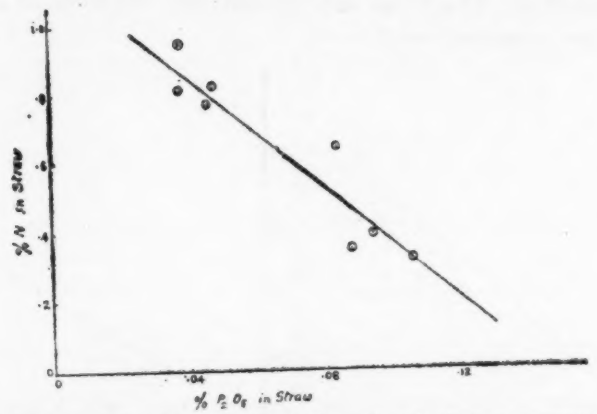

FIG. 3. Inverse relation between phosphoric acid and nitrogen contents of rice straw grown in Kelingon soil.

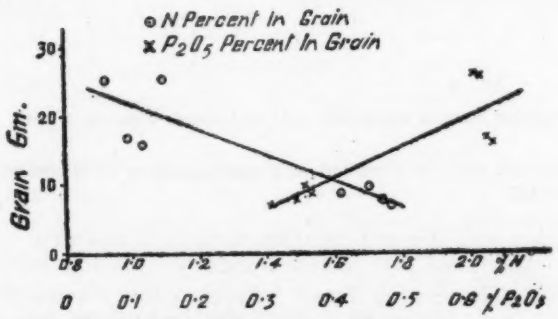

FIG. 4. Relation between yield and composition of rice grain grown in Kelingon soil.

lation of nitrogen content with all the plant characters studied in this experiment was found to be negative and significant. On the other hand, the

various plant characters showed a significant positive correlation with the phosphoric acid content.

Nutrients other than nitrogen and phosphoric acid were determined only in straw. Of these, potassium showed fairly close association with the phosphoric acid content (Fig. 5). The calcium content varied only slightly whereas the magnesium content varied widely. The percentage of magnesium was much higher in the phosphate treated samples than in those grown without phosphoric acid treatment (Fig. 6). The correlation between phosphoric acid content and potassium or magnesium content of straw is shown in Table IX. The percentage of iron in straw was remarkably uniform with only one exception. There was very considerable variation in manganese content but no consistent trend.

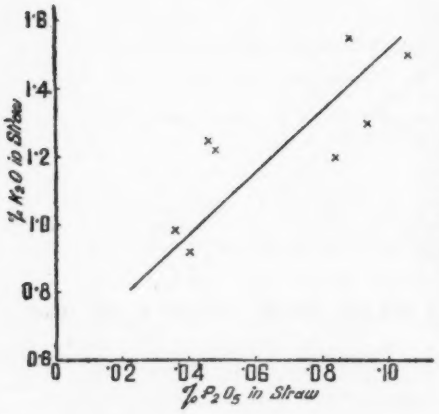

FIG. 5

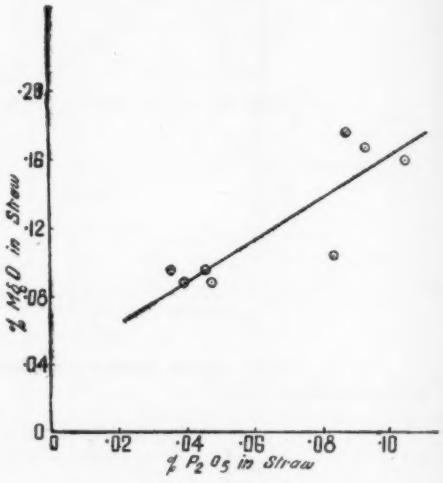

FIG. 6

FIG. 5. Correlation between phosphoric acid and potash contents of rice straw grown in Kelingon soil.

FIG. 6. Correlation between phosphoric acid and magnesium oxide contents of rice straw grown in Kelingon soil.

In connection with this pot experiment observations were made on root development. Additional pots included under certain treatments were used for this purpose. The relative size of the root system compared to the vegetative portion may be seen in Plate VI *a*. The yield and composition of the roots are shown in Table X.

These data show that manuring with phosphate increases the weight of the roots as well as of the tops but the increase of the tops is much greater

TABLE IX

Correlation between P_2O_5 content and other constituents of rice straw and grain grown in Kelingon soil

(Based on Tables IV and VII)

Relationship	Straw %K_2O	Straw %MgO	Straw %N	Grain %N
With phosphoric acid Total ..	5·51	0·608	1·70	4·03
Without phosphoric acid Total ..	4·37	0·368	3·34	6·82
Difference between Totals ..	1·14	0·240	1·64	2·79
Standard error of difference ..	±0·45	±0·066	±0·283	±0·164
t Calculated ..	2·53	3·63	5·78	17·0
Level of significance of difference ..	5%	2%	1%	1%
Correlation with P_2O_5 content *r* ..	+0·805	+0·854	−0·941	−0·989
Standard error of correlation co-efficient ..	±0·242	±0·213	±0·138	±0·06
t calculated ..	3·28	4·01	6·82	16·48
Level of significance of *r* ..	2%	1%	1%	1%

TABLE X

Yield and composition of roots of rice grown in Kelingon soil

Treatment	Dry matter per pot gr.			Composition of root (per cent. on dry matter)			Composition of straw (percentages on dry matter)		
	Root	Top straw	Ratio of root/top	N	P_2O_5	Fe_2O_3	N	P_2O_5	Fe_2O_3
O ..	3·53	8·80	0·40	0·72	0·05	5·54	0·75	0·04	0·12
NK ..	4·41	10·40	0·42	0·79	0·04	5·86	0·92	0·04	0·14
P ..	5·90	18·59	0·32	0·55	0·10	3·25	0·56	0·08	0·11

proportionately. The percentages of nitrogen and phosphoric acid in roots run parallel with the corresponding figures for straw. The very high percentages of iron in the roots are noteworthy. When dried, the roots were of a reddish brown colour in all the treatments employed in the experiment though the colour was much lighter in the case of treatments containing phosphoric acid. In the absence of phosphoric acid there was practically no growth of the bristle-like secondary roots characteristic of normal healthy rice plants. When phosphoric acid was included in the treatment there was a definite tendency for the formation of such roots.

(*b*) *Water culture experiments to study the effects of phosphate deficiency.*— Taungdeikpan seeds were germinated in sand and after 14 days' growth the seedlings were transferred to the appropriate culture solutions. Various

observations were taken with the object of deducing the effects of phosphate deficiency. The experiment was repeated with different concentrations of phosphoric acid in the medium. The data are shown in Table XI.

TABLE XI

Effects of phosphoric acid deficiency on rice in culture solutions

(Average values for 3 bottles)

Experiment Number and Treatment	Concentration of P in p.p.m.	Height of plant cm.	No. of tillers	Length of leaf cm.	Maximum width of leaf mm.	Length of root cm.	Dry weight of tops grams	Dry weight of roots grams	Ratio of Roots/Tops
A—Complete	40	82	12	56	10	19	10·40	3·30	0·32
I/10 P	4	65	7	36	7	28	6·72	2·51	0·38
B—Complete	20	89	12	49	9	22	13·00	3·36	0·29
I/10 P	2	60	4	30	6	28	5·01	2·12	0·42
I/50 P	0·4	38	0	25	3	37	2·20	1·06	0·48
C—Complete	6	90	14	54	10	28	16·40	4·60	0·28
—P	0	34	0	18	3	41	1·54	0·78	0·50

From the data of Table XI it will be seen that a reduction in phosphate supply leads to a reduction in height of plant, reduction in length and width of leaf, and in leaf area, reduction in tillering and in yield of dry matter. The roots however show extreme elongation (Plate VI *b*) and the proportion of roots to the tops increases when the phosphate supply is cut down.

The plants obtained in the solution culture experiments were partially analysed to secure additional information. The results are shown in Table XII.

TABLE XII

Composition of rice plants grown in solution culture with varying phosphate supply

(Samples from B and C of Table XI)

Treatment	Straw (Percentages on dry matter)		Roots (Percentages on dry matter)		
	N	P_2O_5	N	P_2O_5	Fe_2O_3
Complete ..	3·20	1·22	2·01	0·95	3·55
I/10 P ..	3·44	0·91	2·21	0·80	4·82
I/50 P ..	3·65	0·53	3·03	0·48	6·73
— P ..	4·03	0·08	3·39	0·11	8·66

With a reduction in phosphate supply there is a corresponding reduction in phosphate absorption by the plant and a tendency to absorb increasing amounts of nitrogen. The roots also accumulate very large amounts of iron with progressive deficiency in phosphoric acid (Fig. 7).

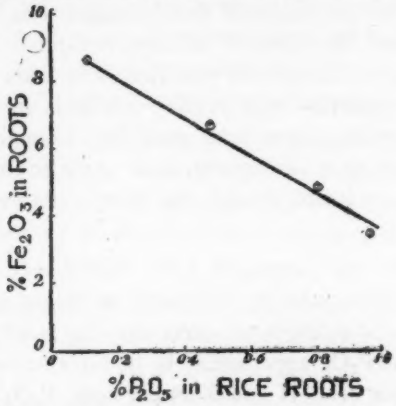

FIG. 7. Accumulation of ferric oxide in rice roots induced by phosphate deficiency in culture solution.

Certain qualitative observations were also made during the course of the experiments. The colour of the plants and the roots suffered very distinct changes when the phosphate supply was reduced. These changes are shown in Table XIII.

TABLE XIII

Appearance of tops and roots of phosphate-deficient rice plants grown in solution cultures

Treatment	Remarks on the appearance of tops and roots
Complete ..	Plants of normal green colour with a small proportion of the older leaves turning yellow. The dried leaves had a pure brown colour. There was no spotting or other discoloration on the green leaves or leaf-sheaths. Roots were of pure white colour with a high proportion of bristle-like secondary roots near the collar.
I/10 P ..	Plants somewhat deeper green with a fair proportion of the older leaves drying with orange yellow colour at the tips. The dried leaves had a greyish brown colour. Some of the older green leaves had numerous brown spots. The older leaf-sheaths were dark brown to nearly black and decayed. In Experiment A (Table XI) the roots were similar to those of the plants grown with full phosphate supply except for the development of a deep brown colour near the surface while the rest of the roots were almost white. In Experiment B, the roots were of a reddish brown colour throughout.
I/50 P ..	Leaves were of bluish green colour and very short and narrow. Most of the leaves were bunched together and stood erect. Leaf drying was similar to that in I/10 P. The dried leaf-sheaths were brownish black and showed symptoms of wet rot. Primary roots were elongated and reddish brown. There were very few secondary roots near the surface.
— P ..	Younger leaves blue green. Older leaves drying to a brown colour. Dark brownish black discolration in patches on the sheath. Roots extremely elongated with a reddish brown colour throughout. Complete absence of secondary roots near the surface.

A deficiency of phosphoric acid therefore not only causes various morphological and colour changes but also renders the plant susceptible to disease.

(*c*) *Pot experiments to compare the effect of different forms of phosphoric acid added to Kelingon soil.*—Having established that phosphoric acid was the major deficiency in the soil and that nitrogen was likely to be useful, the next step was to test the effect of different phosphatic materials with and without added nitrogen. Bonemeal and rice bran were used in this work as these indigenous materials were readily available in Burma even under war conditions. Superphosphate was used for comparison though there was difficulty in obtaining it for general use. As a source of nitrogen only ammonium sulphate was tested though rice bran contained a fair percentage of nitrogen.

A pot experiment was arranged with fresh unmanured soil from Kelingon. The treatments were at the rate of 40 lbs. of nitrogen and 80 lbs. of P_2O_5 per acre. No adjustment was necessary for rice bran as the samples used contained N and P_2O_5 approximately in the ratio of 1:2. Raw rice bran contained 1·90 per cent. N and 3·84 per cent. P_2O_5 and parboiled rice bran contained 1·82 per cent. N and 3·58 per cent. P_2O_5. There were four replications for each treatment and each pot had one seedling. Along with each rice bran treatment an extra set was included in which each pot received in addition 10 c.c. of acriflavine solution (1 part in 500 parts of water) to serve as an antiseptic. The treatments and the corresponding yields are shown in Table XIV.

TABLE XIV

Comparison of phosphatic manures in Kelingon soil

Ser. No.	Treatment	Av. No. of tillers per pot	Straw					Grain				
			a	b	c	d	Mean	a	b	c	d	Mean
1	Nil	5	15·6	18·7	14·9	20·8	17·3	11·7	12·8	10·8	13·5	12·2
2	Super ..	10·5	24·7	23·5	25·6	26·5	25·1	21·8	20·0	22·7	21·1	21·4
3	Super plus $(NH_4)_2SO_4$	15	35·2	30·7	34·2	28·3	32·1	25·6	28·2	28·8	26·6	27·6
4	Raw rice bran	8·5	24·3	18·3	22·7	18·4	20·9	19·5	17·5	18·9	18·1	18·5
5	Raw rice bran plus antiseptic	11·5	28·6	36·2	32·7	28·9	31·6	24·5	30·5	28·6	26·4	27·5
6	Parboiled rice bran ..	11·5	32·1	29·0	26·7	31·4	29·8	27·0	25·1	23·2	28·7	26·0
7	Parboiled rice bran plus antiseptic ..	11·5	30·7	33·7	27·4	29·4	30·3	25·3	28·5	26·0	27·8	26·9
8	Bone meal ..	10	26·5	25·4	29·2	24·5	26·4	22·1	20·4	22·4	23·1	22·0
9	Bone meal plus $(NH_4)_2SO_4$	13·5	32·7	27·8	30·2	28·6	29·8	26·4	22·7	25·0	27·5	25·4

Standard error of the difference between two mean yields of grain = ± 2·46.

Critical value of the difference between two mean yields of grain for significance

(at 1 per cent level = 6·88) (at 5 per cent level = 5·08)

All the treatments are therefore significantly better than the untreated control though some comparisons have proved significant only at the 5 per cent. level. Of the treatments bone meal is as good as superphosphate when used singly but when applied with ammonium sulphate superphosphate appears to be more efficient. The treatments containing nitrogen and phosphoric acid are all of equal efficiency except raw rice bran which however is raised to the same level as the others when accompanied by the antiseptic. This peculiarity is not shown by parboiled rice bran.

(*d*) *Field experiments.*—Preliminary field trials with farmyard manure at 20 cartloads per acre (about 10 tons) rotted paddy chaff at 20 cartloads per acre, and Nicifos 11 at 100 lbs. per acre (about 18 lbs. of P_2O_5) showed that some improvement in crop growth was produced in each case though the increase in yield was negligible.

Based on information obtained in laboratory and pot culture work with Kelingon soil a field experiment was laid down to demonstrate the value of different fertilizers. The treatments were as follows:—

A—Control—No manure.
B—40 lbs. N per acre as ammonium sulphate.
C—40 lbs. P_2O_5 per acre as bone meal.
D—40 lbs. P_2O_5 per acre as superphosphate.
E—Ammonium sulphate plus bone meal at the same rates.
F—Ammonium sulphate plus superphosphate at the same rates.

The treatments were arranged in the form of a Latin square with suitable randomisation. Each plot had an area of 22 × 33 sq. ft. or 1/60 acre. The layout and the yields are shown in Table XV and the statistical treatment in Table XVI.

TABLE XV

Kelingon field experiment

(Grain yields in lbs. per plot)

	1	2	3	4	5	6	Rows–Totals
1	A 14·0	F 18·6	D 12·4	E 16·9	B 15·3	C 26·3	103·4
2	D 26·3	C 15·5	A 5·6	B 12·7	E 18·7	F 29·4	108·2
3	B 16·8	D 15·1	E 29·9	F 28·3	C 20·4	A 14·2	124·7
4	C 29·3	E 18·3	F 17·1	D 21·9	A 12·2	B 9·5	108·3
5	F 17·5	B 7·8	C 17·6	A 8·4	D 12·6	E 28·3	92·2
6	E 22·8	A 16·5	B 10·9	C 10·0	F 28·4	D 24·9	113·5
Columns—Totals ..	126·7	91·8	93·5	98·2	107·6	132·5	Grand Total 650·3

Treatments

(Replicates and Totals)

Replicates	A	B	C	D	E	F
1	14·0	15·3	26·2	12·4	16·9	18·6
2	5·6	12·7	15·5	26·3	18·7	29·4
3	14·2	16·8	20·4	15·1	29·9	28·3
4	12·2	9·5	29·3	21·9	18·3	17·1
5	8·4	7·8	17·6	12·6	28·3	17·5
6	16·5	10·9	10·0	24·9	22·8	28·4
Yield Totals	70·9	73·0	119·0	113·0	134·9	139·3

TABLE XVI

Analysis of variance of grain yields shown in Table XV

Sources	Degrees of freedom	Sum of squares	Variance	F calculated	F from tables for P = 0·01
Rows ..	5	96·53	19·30		
Columns ..	5	252·99	50·60		
Treatments ..	5	741·98	148·40	5·17	4·10
Error ..	20	574·02	28·70		

The effect of treatments is therefore highly significant.

Standard error of the difference between two yield totals = ± 18·56.

Critical difference fot significance at 5 per cent. level = 31·72 lb.

The yield totals fall into three contrasted groups, namely (i) E and F, (ii) C and D, and (iii) A and B, but groups (i) and (ii) do not differ significantly from each other. Treatments A and B which do not contain phosphoric acid are significantly inferior to each of the other treatments. It would appear therefore that the effect of the addition of nitrogen to phosphoric acid may not be significant though the large differences shown by treatments E and F over C and D are suggestive. A higher rate than 40 lb. of nitrogen per acre for manuring rice is not likely to be accepted in practice even if it produced a significant increase in yield.

The straw yields as well as the chemical analysis data are included in Tables XIX and XX under Discussion.

Crop samples from other field experiments conducted at Kelingon were analysed and the data are shown in Table XVII.

The composition of the field samples agrees generally with that of the pot culture samples. In the absence of phosphoric acid the crop is poor

TABLE XVII

Composition of mature rice straw and grain grown on Kelingon soil

(Oven dry basis)

Serial No.	Treatment	Straw		Grain	
		N per cent.	P_2O_5 per cent.	N per cent.	P_2O_5 per cent.
	Manuring Experiment				
1	Control-unmanured ..	0·662	0·033	1·246	0·216
2	Nicifos II at 30 lb. P_2O_5 per acre ..	0·406	0·069	1·068	0·564
3	Superphosphate ,, ,, ,, ..	0·350	0·065	0·970	0·553
4	Bone meal ,, ,, ,, ..	0·392	0·063	1·016	0·556
5	Bone meal at 60 lbs. ,, ,, ..	0·390	0·076	0·980	0·608
	Liming Experiment				
1	Control-untreated	0·692	0·038	1·248	0·196
2	5 cwt. slaked lime per acre ..	0·604	0·044	1·248	0·232
3	10 cwt. ,, ,, ,, ..	0·648	0·046	1·220	0·276
4	20 cwt. ,, ,, ,, ..	0·660	0·033	1·273	0·260
5	40 cwt. ,, ,, ,, ..	0·760	0·030	1·270	0·228
6	80 cwt. ,, ,, ,, ..	0·928	0·028	1·344	0·202

in this constituent but is abnormally rich in nitrogen. These two features are corrected by phosphatic manures.

Although Kelingon soil is very acidic liming does not improve the crop as may be judged by the composition of grain and straw. Observation in the field showed that increase in the rate of liming caused an increase in the depth of green colour which was bluish green for the 80 cwt. rate. This change corresponds with the depression in P_2O_5 content and the increase in N content of the crop. Small doses of lime however appear to mobilise the available P_2O_5 but the effect is too small to be of practical value.

DISCUSSION

(i) *Plant size.*—The data of Tables II and XI demonstrate that phosphate deficiency causes reduction in height, leaf size, leaf area, and tillering in rice. Kellner (1890) noted the reduction in tiller formation, while Warth and Po Shin (1919) recorded a reduction in the " volume " of the rice plant in soils deficient in phosphoric acid. Sircar and Sen (1941) observed in sand culture that phosphate deficiency caused a reduction in height and in tiller formation in rice. The effects of phosphate deficiency have been studied in barley by Brenchley (1929) and by Richards and Templeman (1936), in oats by Williams (1936), and in sugarcane by Martin (1934) and by Saito

and Kenjo (1939). On the whole, rice is similar to other cereals in regard to the effects of phosphate deficiency on morphological features.

(ii) *Colour.*—A deficiency of phosphoric acid is known to cause noteworthy symptoms in plants. A dark green colour has been noted in barley by Richards and Templeman (1936), in tobacco by McMurtrey (1938), in cotton by Cooper (1939), and in white pine seedlings by Mitchell (1934). On the other hand, Suzuki (1908) found that the stems of phosphate starved barley had a red colouration while the leaves were purple coloured. Meyer (1930) found that tomato seedlings develop a purple colour on the stems and lower sides of the leaves within ten days after germination when grown in a phosphate-deficient medium. This colour reaction is so sensitive that the tomato plant has been recommended as a reliable indicator for phosphate deficiency (Meyer, 1930; McDonald, 1933). Leaves of certain fruit trees also show similar anthocyanin pigmentation when they are subjected to phosphate deficiency (Hoagland and Chandler, 1932).

In the present work it has been found that phosphate-deficient rice plants have a blue green or dark green colour (Tables III and XIII). This colour becomes noticeable about four to six weeks after germination and persists until the date of harvest n sharp in contrast with the golden yellow colour of the phosphate treated mature plant. Warth (1916) has remarked on the dark green colour of rice plants grown in phosphate-deficient soils especially when manured with ammonium sulphate. Although the dark green colour of the plant is very striking it is not distinctive as it has been found (Aiyar, 1945 *a*) that sulphate deficiency gives rise to similar symptoms.

(iii) *Root development.*—Phosphate manuring is believed to cause an increased development of the root system particularly in clay soils. Apparently this view originated with the investigations at Rothamsted on the development of root crops (Russell, 1942) and seems to have been assumed to hold good for all crops.

Goedewaagen (1937) showed that a high concentration of phosphate in the growth medium does not always stimulate root development, but that on the other hand, the beneficial action of phosphate arises from an increase in the shoot-root ratio of plants.

Ramiah's view (1937) that phosphatic and potassic fertilizers do not seem to have any particular effect on root development of rice and that their application without nitrogenous manures may even inhibit the growth of roots appears to hold good only for soils with nitrogen deficiency.

When grown in phosphate-deficient solution the rice plant has now been found to develop a peculiar root system which (i) becomes elongated with progressive deficiency of phosphoric acid (Table XI); (ii) is devoid of the bristle-like secondary roots; (iii) is of a deep brownish red colour and contains a high percentage of iron (Table XII); and (iv) bears a higher proportion by weight to the vegetative portion compared to the phosphate-treated plants (Tables X and XI). The roots of plants grown in the phosphate deficient Kelingon soil showed similar symptoms except that elongation was not appreciable. The difference between solution culture and soil culture in causing root elongation in rice may be partly accounted for by the unavoidable fact that in a soil the root development is limited to the puddled layer whereas in a solution no such mechanical restriction exists. Other possible factors that might account for this difference may be suggested: (*a*) the culture solution contained nitrate-nitrogen in addition to ammonium-nitrogen whereas the soil did not contain nitrate in any appreciable amount, (*b*) the quantities of manganese and iron present in the culture solution were strictly limited whereas in the soil these two elements might have been available in much larger (or smaller) amounts and in a different proportion. In the absence of sufficient phosphoric acid the respiratory activity and excretion of carbon dioxide by the roots may be greatly reduced (Lyon, 1927) and thereby the plant may not be able to obtain enough manganese or some other essential element; Chapman (1941) has observed a case of induced manganese deficiency in citrus grown in a phosphate-deficient medium; (*c*) poor aeration is another factor that might restrict root growth of rice in phosphate-deficient soil; Vlamis and Davis (1943) have shown that root growth of rice is greatly depressed while shoot growth is appreciably increased by a reduction in oxygen supply within the growth medium. In soil it is practically certain that phosphate deficiency prevents the growth of algæ which are regarded as the main aerating agents in rice fields (Harrison and Aiyer, 1914).

Although the author has found in solution culture that a deficiency of many of the major nutrients causes elongation of the root system of rice the effect is particularly striking in cases of phosphate deficiency and of sulphate deficiency (Plate VI *b*). In both these cases the roots are extraordinarily long but they are pure white in sulphate deficiency (Aiyar, 1945 *b*) while they are deep reddish brown in phosphate deficiency, a very distinctive feature.

(iv) *Yield of crop.*—The low yield from the unmanured pots (Table IV) and from the unmanured field (Table XV) shows that the Kelingon soil is in a poor condition from which a satisfactory yield of crop cannot be

expected without appropriate manuring. The addition of nitrogen and potash individually produces no noticeable effect whereas the joint application depresses grain yield though the straw yield is not affected. The application of phosphoric acid alone or with potassium sulphate almost doubles the yield. One may thus conclude that Kelingon soil is very deficient in phosphoric acid and that unless this nutrient is added no increase in yield can be expected in accordance with the Law of the Minimum. When however phosphoric acid is applied jointly with nitrogen the yield is raised still further though the inclusion of potash as well shows no additional benefit. The conditions for obtaining maximum yield from this soil were investigated and it was found in pot experiments that 80 lbs. of P_2O_5, 40 lbs. of N, or 20 lbs. of K_2O were sufficient to produce a maximum crop when applied in presence of a basic treatment of 80 lbs. of each of the other two nutrients. These rates are too high to be adopted by cultivators. It would appear therefore that if economic considerations are allowed to dominate production an application of bone meal at 2 cwt. per acre would be the only safe recommendation. Parboiled rice bran might also be recommended but an application of about half a ton will be necessary to provide 40 lbs. of P_2O_5 per acre.

Phosphoric acid is regarded as essential for grain formation but obviously this nutrient is equally essential for straw growth as well. Although phosphate deficiency causes a low yield of crop in Kelingon soil there is no tendency for the grain to be chaffy. The straw-grain ratio does not vary appreciably with treatment as most of the ratios lie between 1·0 and 1·25 (Tables IV and XIX).

(v) *Composition of the crop.*—Kellner *et al.* (1890) found that the soil used by them gave large responses to phosphate manuring and that in the absence of phosphoric acid in the manure the percentage of this constituent in straw was low though the corresponding grain had the normal percentage of phosphoric acid. Warth (1916) and Warth and Po Shin (1919) emphasize the low phosphoric acid content of rice straw grown in phosphate deficient soils but apparently their grain was normal as they do not remark on its composition. It is generally accepted that the composition of grain is less readily affected than that of the straw. However both the straw and grain of the crop produced in the unmanured Kelingon soil contain an extremely low percentage of phosphoric acid (Table VII) which is raised only when phosphoric acid is included in the manures applied. The fact that the phosphoric acid content of the unmanured rice grain is only about one half of the value found in the grain manured with phosphoric acid should be taken

as an indication of the existence of an acute deficiency of phosphoric acid in Kelingon soil as distinct from a relative deficiency.

The observation of Lagatu and Manme (Thomas, 1929) that an unbalanced fertilizer applied to a crop causes a depression in the absorption of the "missing" element is illustrated by the figures for P_2O_5 in rice straw manured with N or NK (Table VII). Warth and Po Shin (1919) have drawn attention to the same fact. These authors as well as Kellner *et al.* (1890) attribute the depression in P_2O_5 content of straw caused by manuring with N or NK to the better utilization of phosphoric acid by the plant. This explanation does not appear to be of general validity because treatment with NK has not produced an increased yield compared to the unmanured crop (Table IV).

A characteristic effect of phosphate deficiency on rice is the heavy accumulation of nitrogen in straw as well as in grain. This effect is easily recognised as the nitrogen content of the phosphate manured plant is much lower than that of the plant receiving no phosphate. In pot culture the straw manured with pure orthophosphoric acid contained a fairly high percentage of nitrogen, possibly due to limited supply of sulphate in the soil (Aiyar, 1945*a*). When fortified with $CaSO_4$ treatment with H_3PO_4 did not cause an increase in yield compared with H_2PO_4 alone. The mean percentages of nitrogen are 0·425 in straw and 1·08 in grain of phosphate-treated plants whereas in plants not treated with phosphoric acid the nitrogen figures are 0·835 in straw and 1·705 in grain. These differences were found to be highly significant (Table IX).

There is a considerable volume of opinion in regard to the effect of phosphate deficiency on the nitrogen metabolism of various plants. The author obtained some data on the nitrogen and carbohydrate fractions in rice plants grown in Kelingon soil. These data are shown in Table XVIII.

TABLE XVIII

Nitrogen and carbohydrate fractions of rice plants sampled on 15th October 1940

(Percentages on dry matter)

Treatment	Total N	Protein N	Soluble N	Soluble carbohydrates as glucose	Dextrin and Starch
O ..	1·120	0·741	0·379	8·11	2·66
NK ..	1·942	0·876	1·066	12·71	2·83
P ..	0·534	0·389	0·145	4·11	2·41

The accumulation of total N, protein N and soluble N in the absence of phosphate treatment is very striking. The observation in regard to soluble nitrogen (amino-acids and acid amides chiefly) agrees with the findings of other investigators (Richards and Templeman, 1936; Chapman, 1938). In respect of total nitrogen there is agreement with certain published data (McGillivray, 1927; Saito and Kenjo, 1938) but the author's observation in regard to the accumulation of total nitrogen as well as protein nitrogen in phosphate-starved rice is entirely opposed to the findings of Sircar and Sen (1941). The disagreement may be attributed to the possibility that the culture solution used by Sircar and Sen (*loc. cit.*) was unsuitable for rice, although suited for barley. Actually, the solution used by these authors was a slightly modified form of the mixture used in work on barley (Gregory and Richards, 1929). It is known (Kimura, 1931) that barley can tolerate a much higher concentration of phosphoric acid than rice, and accordingly the full dose of phosphoric acid used by Sircar and Sen probably proved harmful to the plant. This view is strengthened by the fact that no earheads were produced when the solution contained the full dose of phosphoric acid and that when the dosage of this nutrient was reduced to a small fraction of the maximum concentration all the plants produced earheads. In view of this result it is doubtful if phosphate starvation existed in their solution even at the lowest level tested by them. It may be seen from Table XI that maximum yields are obtainable with a phosphorus concentration of 6 p.p.m. in the solution.

Gregory and Baptiste (1936) found that phosphate deficiency in barley caused a reduction in soluble sugars while other investigators (Kraybill and Smith, 1924; McGillivray, 1927; and Kraybill, 1930) have found a high percentage of sugars in tomato grown under phosphate deficiency. It has now been found that phosphate-deficient rice contains a much higher percentage of soluble sugars than the phosphate treated plants. In regard to starch and dextrin there is no difference between the two sets of plants. The accumulation of sugars may be explained by the poor growth of the plant and by the low rate of respiration due to the shortage of phosphate supply.

The relation of phosphate deficiency to other ash constituents in straw was also examined. As pointed out in connection with Tables VII and IX there was no consistent trend in the percentages of calcium, iron or manganese but the percentages of potassium and magnesium were found to be significantly correlated with the phosphoric acid content. In both cases manuring with phosphoric acid caused a significant increase in the absorption of these two nutrients. Russell (1943) has drawn attention to the phosphoric acid-potash relationship in plants. Kenjo and Saito (1939) found that

phosphate-deficient sugarcane contained much less potash than fully manured plants.

(vi) *Efficiency of phosphatic manures.*—In general soluble phosphates are the only ones regarded as universally applicable. Bone meal and organic phosphatic materials are unsuitable for many soils as these react too slowly. In Kelingon soil, however, all these three types of phosphatic materials were found to be equally satisfactory sources of phosphoric acid as judged by the yield of crop and the percentages of phosphoric acid and nitrogen in the crop (Tables IV, VII, XIV, XV and XVII).

Kellner *et al.* (1890) found an average recovery of about 20 per cent. of phosphoric acid for the range of application of 45 pounds to 180 pounds of phosphoric acid per acre. Haigh and Joachim (1934) obtained a recovery of 11 per cent. with an application of 36·5 pounds of phosphoric acid per acre. In pot experiments the author obtained recoveries up to 50 per cent. while in the field the highest recovery was 16 per cent. In each case, the improved recovery was due to increased percentage as well as increased yield (Table XIX).

TABLE XIX

Recovery of phosphoric acid by rice from Kelingon soil

(Based on Tables IV, V and XV)

Treatment	Straw			Grain			Total P_2O_5 absorbed g.	Diff. from control g.	Recovery of added P_2O_5 %
	Yield g.	P_2O_5	P_2O_5 absorbed	Yield g.	P_2O_5	P_2O_5 absorbed g.			
Pot Experiment									
0·3 g. P_2O_5 pot per = 80 lb. per acre									
O ..	8·9	·046	·0041	8·7	·365	·0318	·0359	..	..
N ..	10·0	·038	·0038	9·9	·355	·0351	·0389	·0030	..
NK ..	9·5	·038	·0036	6·9	·305	·0210	·0247	−·0112	..
P ..	18·8	·084	·0158	16·0	·636	·1018	·1176	·0817	27·2
PK ..	21·0	·106	·0223	16·7	·626	·1045	·1268	·0909	30·3
NP ..	24·4	·094	·0229	25·2	·613	·1545	·1774	·1415	47·2
NPK ..	31·8	·088	·0280	25·7	·618	·1588	·1868	·1509	50·3
Field Experiment									
40 lb. P_2O_5 per acre									
	lb.		lb.	lb.		lb.	lb.	lb.	
O ..	793	·036	0·285	709	·402	2·850	3·135	..	
N ..	757	·034	0·258	730	·388	2·832	3·090	−0·045	
P (Bone meal)	1287	·087	1·104	1190	·567	6·747	7·851	4·716	11·8
P (Super) ..	1371	·091	1·248	1132	·580	6·565	7·813	4·678	11·7
P (Bone meal) plus N ..	1483	·089	1·320	1349	·612	8·256	9·576	6·441	16·1
P (Super) plus N ..	1501	·088	1·321	1393	·593	8·260	9·581	6·446	16·1

The recoveries fall into three distinct groups: (*a*) no phosphoric acid, (*b*) phosphoric acid without nitrogen and (*c*) phosphoric acid with nitrogen, the highest recovery being obtained in the last case. The efficiency of phosphoric acid is therefore greatly improved by the addition of nitrogen. Even

TABLE XX

Recovery of Nitrogen by rice plant grown in Kelingon soil

(Based on Tables IV, V and XV)

Treatment	Straw			Grain			Total N absorbed g.	Diff. from control g.	Recovery of N added %
	Yield g.	N	N absorbed g.	Yield g.	N	N absorbed g.			
Pot Experiment									
0·3 g. N per pot = 80 lb. per acre									
O ..	8·9	·77	·0685	8·7	1·62	·1409	·2094	..	..
N ..	10·0	·81	·0810	9·9	1·70	·1683	·2493	·0399	13·3
NK ..	9·5	·94	·0893	6·9	1·76	·1214	·2107	·0013	0·4
P ..	18·8	·64	·1203	16·0	1·03	·1641	·2851	·0757	..
PK ..	21·0	·32	·0672	16·7	0·92	·1516	·2208	·0114	.
NP ..	24·4	·39	·0953	25·2	1·09	·2747	·3700	·1606	53·1
NPK ..	31·8	·35	·1113	25·7	0·99	·2544	·3657	·1563	52·5
Field Experiment									
40 lb. N per acre									
	lb.		lb.	lb.		lb.	lb.	lb.	
O ..	793	·693	5·495	709	1·283	9·096	14·59	..	..
N ..	757	·721	5·458	730	1·311	2·570	15·03	0·44	1·1
P (Bone meal)	1281	·403	5·161	1190	1·038	12·352	17·51	2·92	..
P (Super) ..	1371	·391	5·361	1132	1·046	11·875	17·24	2·65	..
P (Bone meal) plus N ..	1483	·382	5·665	1349	0·988	13·328	18·99	4·40	11·0
P (Super) plus N ..	1501	·388	5·824	1393	0·995	13·860	19·68	5·09	12·7

the highest recoveries found in the field experiment are very low compared to those obtained in the pot experiment. The large difference in recovery between the pot experiment and the field experiment might be partly due to the higher rate of application in the pot (80 lbs. P_2O_5 per acre) and partly due to the different methods of placement. In the field the manure was scattered on the surface and harrowed into the mud whereas in pots the fertilizer was merely distributed on the surface.

In view of the dependence of phosphoric acid on applied nitrogen for greater efficiency the recovery of nitrogen was also calculated for comparison. The results are shown in Table XX.

From the data of Table XX it will be seen that a high recovery of nitrogen is possible only when phosphoric acid and nitrogen are applied together.

The relatively low recovery of nitrogen in the field experiment is on the same level as that of the recovery of phosphoric acid. The recovery of 62 per cent. obtained by Kellner *et al.* (1890) for a range of application of 45 lbs. N to 90 lbs. N per acre and a recovery of 70 per cent. for an application of 9·3 lb. per acre by Haigh and Joachim (1934) suggest the possibility that direct losses or conversion to non-available forms might be responsible for the low recoveries of added nitrogen from Kelingon soil.

The results of pot and field experiments discussed here should serve as a warning against the application of nitrogenous manures until it is ascertained that the soil is not deficient in phosphoric acid or in some other nutrient.

Summary

1. The experiments described here were carried out in pots and in the field with Kelingon soil found to have a low phosphate content to study the effects of phosphate deficiency on rice. Experiments in solution culture confirmed the observations made in the soil.

2. Rice plants grown under phosphate deficiency were stunted compared to the phosphate manured plants. The phosphate-deficient plants showed reduction in height, in length and width of leaves, as well as in tiller formation. The leaves showed a tendency to remain bunched together. Compared to the fully manured plants the phosphate-deficient plants had a dark green or blue green colour.

3. A very characteristic feature observed in solution culture was the extraordinary elongation of the primary roots which were of a deep reddish brown colour in contrast to the white colour of the roots of fully manured plants. Another feature was the complete absence of secondary surface roots. Rice plants grown in phosphate-deficient Kelingon soil had roots which did not show appreciable elongation but were similar in other respects to those produced in solution culture. On the whole the difference between roots of phosphate-deficient rice plants and those of fully manured plants grown in Kelingon soil consisted in a large reduction in volume under the deficiency.

4. Manuring the soil with phosphoric acid produced a large increase in yield and a further moderate but significant increase resulted when phosphoric acid was applied together with nitrogen. There was no response to nitrogen or potash individually or jointly until phosphate was also added.

5. In the absence of phosphoric acid the crop failed to reach maturity and remained green at the normal time of harvest, though ear formation and grain development proceeded normally.

6. Responses were equally good whether phosphoric acid (P_2O_5) was applied in the form of pure orthophosphoric acid, superphosphate, bone meal or parboiled rice bran. The effect of raw rice bran was low but was greatly improved by adding to the soil a small quantity of acriflavine as an antiseptic.

7. Manuring with phosphoric acid caused a large increase in absorption of this nutrient by the crop. The crop grown in unmanured soil or in soil manured without phosphoric acid contained abnormally low percentages of phosphoric acid in straw as well as in grain, an indication of acute deficiency.

8. In the crop grown without phosphoric acid the nitrogen content of the straw as well as of the grain was abnormlaly high whereas the fully manured crop contained the normal percentages of nitrogen. The straw btained in pot culture by manuring with orthophosphoric acid alone contained a fairly high percentage of nitrogen, possibly due to the limited supply of sulphate present in the soil. Phosphate deficiency was also found to cause an accumulation of total protein and soluble nitrogen in the plant.

9. In the composition of straw grown in Kelingon soil under different treatments a significant positive correlation was found between phosphoric acid content on the one hand and potassium or magnesium content on the other. The correlation between nitrogen and phosphoric acid contents of straw as well as of grain was negative and highly significant.

10. Rice roots grown under phosphate deficiency contained a much higher percentage of iron compared to roots of plants manured with phosphate.

11. Phosphate deficiency led to a poor recovery of added nitrogen by the crop due to poor growth. The recovery was greatly improved by phosphate manuring though maximum efficieney was attained only by a joint application of phosphoric acid and nitrogen.

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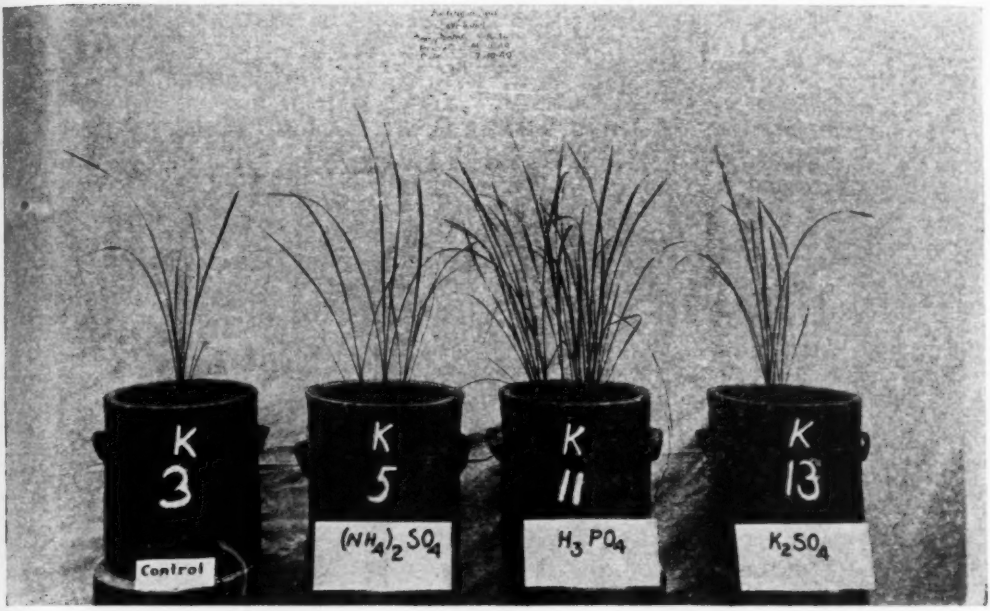
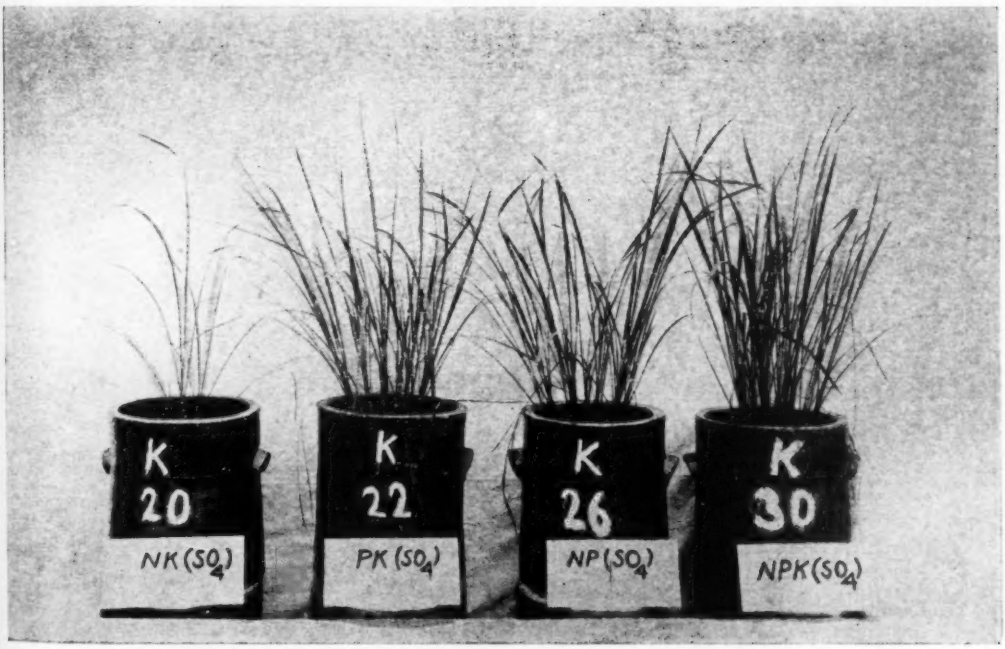
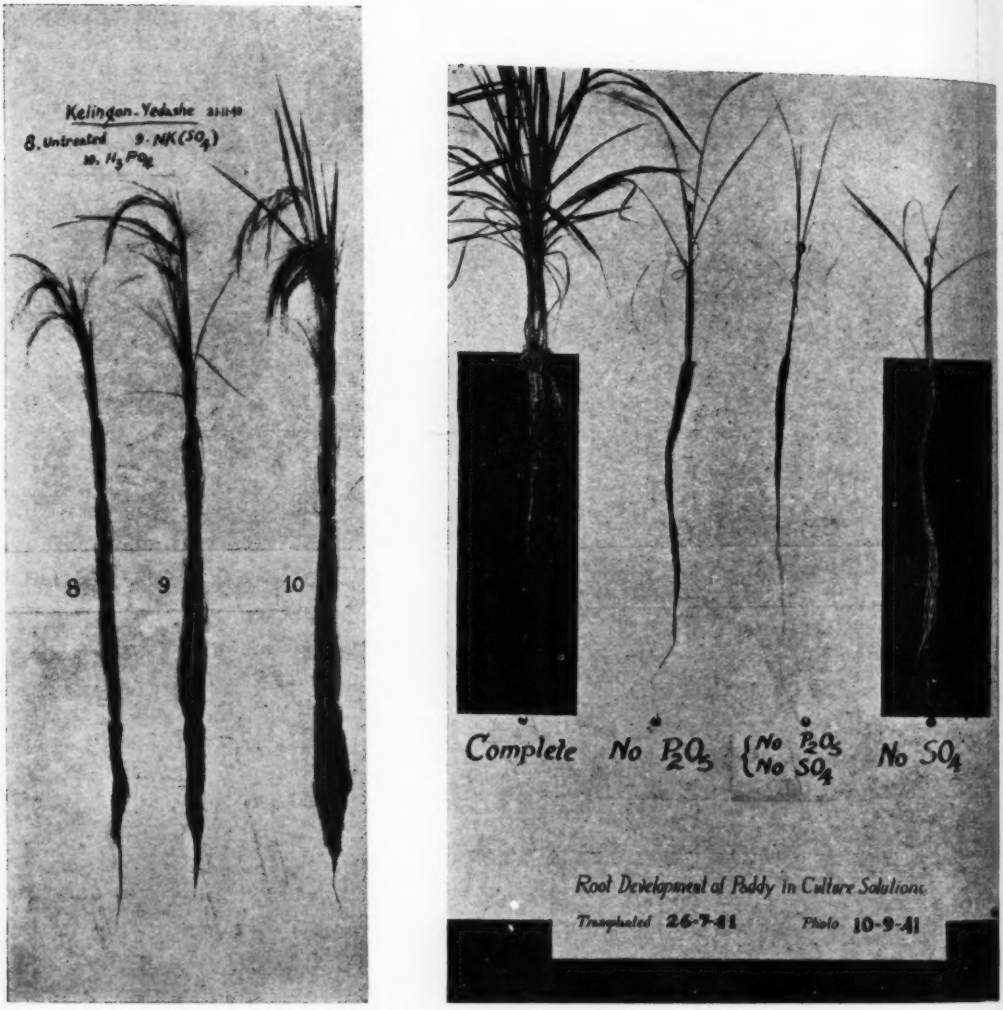




a and *b*. Response of Rice to Nutrients added to Kelingon Soil

a b



a. Effects of Phosphate Treatment on Development of Rice in Kelingon Soil

b. Effects of Phosphate and Sulphate Deficiency on Root Development of Rice in Culture Solutions

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RANDOM SAMPLING FOR ESTIMATING RICE YIELD IN KOLABA, BOMBAY

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During the last two years the Statistical Section of the Imperial Council of Agricultural Research has carried out several large-scale sample surveys for estimating the yield per acre and the total out-turn of wheat and rice. The first survey was carried out on wheat in 1943–44 in the provinces of the Punjab and the U.P. A report containing the results of this survey in the Punjab has appeared in a previous number of these *Proceedings*.[1] A brief account has also appeared in *Nature*.[2] During the year 1944–45, the survey was extended to the whole of India's wheat belt comprising of the provinces of Sind, North-West Frontier Province, Punjab, United Provinces and the Central Provinces. In the current season the survey has been extended to all the rice-growing districts of the United Provinces, Central Provinces, Bombay, Madras, Orissa and Bihar.

2. As a preliminary to extending the surveys to paddy crop, pilot surveys were conducted in the last season in one district each of the provinces of Bombay, Central Provinces and Madras with the objects of (*a*) testing the applicability of the technique developed for wheat for estimating the yield per acre of paddy, and (*b*) for gaining information on the practical difficulties peculiar to paddy crop likely to be encountered in field work. The results of the pilot survey conducted in the Tanjore District of the Madras Province, and their bearing on the technique of random sampling are described elsewhere. The results of the survey conducted in the Kolaba District of the Bombay Province form the subject of this paper.

3. The district of Kolaba was selected for the scheme as typical of the paddy-growing tract of the Bombay Province. It is divided into seven taluks covering an area of 2,165 sq. miles. Of the total cultivated area in the district approximately 67 per cent. is under paddy. It receives ordinarily a rainfall of over 80 inches. It is for the most part a hilly tract containing 1,556 villages.

4. The survey was conducted under the technical direction of the Statistical Adviser, Imperial Council of Agricultural Research, New Delhi, and under the administrative control of the Director of Agriculture exercised through the Deputy Director of Agriculture (Crop Research). The Technical Assistant to the latter was the officer in charge of the training and inspection. The field staff consisted of 7 Agricultural Assistants and 7 Kamgars at the rate of one Agricultural Assistant and one Kamgar for each of the 7 taluks of the district. All the Agricultural Assistants were men with considerable practical training in agriculture and had some knowledge of the tract and of the rice crop.

5. The plan adopted for the survey was the stratified plan of random sampling with taluks as the strata, early, mid-late and late varieties as the sub-strata, a village as the primary unit of sampling, a field as the sub-unit of sampling, and one-guntha plot as the ultimate unit of sampling. The three substrata were defined as follows according to the local conventions :

Early crop : Crop maturing about the 15th of October;
Mid-late crop : Crop maturing between the 16th and 25th of October ;
Late crop : Crop maturing after 25th October.

A total of 84 villages representing approximately 5·4% of the total number of villages in the district was selected. These were divided equally among the 7 taluks. Within each taluka 3 villages were selected for early crop, 5 for mid-late crop and 4 for the late crop. The villages were selected at random from amongst all villages in the taluks separately for the early, mid-late and late strata, so that every village got the same chance of being included in the 3 sub-strata in the taluk. The selection was made with the help of printed random numbers to ensure equal chance for every village in the taluk. The selection was done personally by the Statistical Adviser since he anticipated that considerations of distance and lack of communications and other difficulties might weigh with the provincial staff and be allowed to interfere with the random character of the sample.

6. In villages selected for 'Early Crop', experiments were conducted on early paddy only; in villages selected for 'mid late' crop experiments were conducted on mid-late varieties only ; and similarly in villages for 'late crop' experiments were conducted on late varieties. In each village, 3 fields from amongst all those growing paddy of the particular description were selected at random, and within a selected field a plot measuring $66' \times 16\frac{1}{2}'$ (1/40th of an acre) was located at random. Thus in a village belonging to the early crop, the Agricultural Assistant was required to select 3 paddy

fields at random from amongst all the early paddy fields in the village ; if a village belonged to mid-late crop, the Assistant was required to select 3 paddy fields growing mid-late variety from amongst all mid-late paddy fields in the village ; and so on. As there was a possibility that a village might not have the requisite number of fields of the given description, additional villages were given. If one or more of the villages did not contain the requisite number of fields of the particular description, they were to be rejected and substituted one by one by the villages in the supplementary list in the order given.

7. A detailed set of instructions was drawn for the guidance of the field staff for (*a*) selection of fields, (*b*) location of plots, (*c*) harvesting and connected operations, and (*d*) driage. The fields were selected in advance, to ensure that all the fields in the villages got an equal chance of being sampled. The plots in the selected fields were however marked only on the dates on which the crop was harvested to ensure that the plots were not tampered with. All the operations of harvesting, threshing, winnowing and weighing were carried out by the Agricultural Assistants assisted by field men, and were supervised by the Officer-in-Charge.

8. Four forms of returns were prescribed under the instructions. In Form No. 1, the Agricultural Assistant with the assistance of the Patwari concerned, is required to show the survey numbers and areas of all the paddy-growing fields in the selected village, arrange them serially, and to select 3 fields out of these by using the random numbers supplied to him. In case the number of fields was large, the Agricultural Assistant was asked to select three fields directly from the Patwari's Register with the survey numbers corresponding to the random numbers selected by him. In form 2 of the returns, he is required to give in respect of the three selected fields the pair of random numbers selected for locating plots, the type and level of the soil, preceding crop, manure in current season, the variety of paddy, the estimated yield of paddy per acre, and finally the date fixed for harvesting. The Agricultural Assistant was also asked to give detailed remarks in regard to the general condition of the crop. Both the forms 1 and 2 were required to be sent to the Statistical Adviser immediately after the selection was over. Under form No. 3 the Agricultural Assistant was required to fill the actual results of harvesting in respect of each plot in the village. The Agricultural Assistant was specially asked to note any changes in the condition of the crop that may have taken place since the fields were selected. The return was required to be sent immediately after the harvesting in each village was over. Form No. 4 sets out the results of driage.

9. The scheme commenced on the 1st of September 1944. The first fortnight was spent in preliminary arrangements, *e.g.*, planning, the training of the field staff, arranging for help from the revenue officials, etc. The training of the field staff commenced on the 15th of September and continued for a week. As the entire success of such a survey depends on the reliability of field work and consequently on the training received by the staff, the greatest attention was paid to the question of the training of field staff. Firstly, the field staff were gathered at the Rice Breeding Station, where the plan of work, the duties and responsbilities of each memberof the field staff, the meaning and implications of the instructions drawn for the actual conduct of field work, the manner in which the returns were to be filled in and despatched, were read out, discussed and explained at length several times over, until the workers, particularly the Agricultural Assistants, obtained a clear idea of the entire procedure of work. It was impressed on them that the work they were doing was of great importance, especially during the emergent food situation at the present time and demanded extreme scientific honesty. The training was completed with practical exercises in the fields of the Rice Breeding Station and of the cultivators round about the farm. The fields chosen were of various categories, including bunded fields, of different sizes and shapes, irrigated and unirrigated, so that the staff could have a thorough grounding in the intricacies of the work. Before they dispersed, the workers were provided with a cross-staff, a 100-feet measuring tape, coir strings, scales and weights, bamboo sticks, two threshing durries, and the requisite number of copies of various returns and the sets of random numbers. They were advised to halt for a day or two at the taluka centres, get acquainted with the revenue staff (*e.g.*, the Circle Inspectors, Talatis), and obtain general information about the villages selected for work and the routes thereto.

The training classes were also attended by prominent public workers of the district who had been for some time pressing their point of view that the district did not grow as much rice as was made out in the official forecast, and who therefore were interested in this new method of estimating the actual production.

10. After the staff returned to their respective talukas they proceeded to locate villages one by one. The villages selected were scattered over the taluka as they should be in the random selection. Some of them lay very far off from the only main road in the province, on which there is a bus service, and a few were even at prohibitive heights on mountain slopes. Even though the staff knew the routes to the selected villages, they found it

pretty difficult, on account of the scanty and unsatisfactory communication, heavy rain, and slippery paths, to reach the villages in proper time. As the scheme was prepared at very short notice, and as the harvesting of the early crop was about to commence, there was not even time to write to the Patwaris of the selected villages to ascertain whether the selected villages had the requisite number of fields of the given description. Consequently the Agricultural Assistants themselves had to visit villages one by one in order to ascertain whether there were the requisite number of fields. A large number of villages in the early crop had to be rejected, and then substituted by villages in the supplementary list, because the early crop, contrary to the unconfirmed impression, made only an insignificant portion of the whole rice area in the district. Even villages in the supplementary list had to be rejected. As the harvesting of the early crop had already commenced, the work could be carried out in only 12 out of the 21 villages allotted to the early crop. No difficulty was, however, experienced in locating the villages under mid-late and late crops.

11. Out of a total of 225 fields (3 in each of the 75 villages) selected under the scheme, experiments could be performed in 223. In the remaining 2, owing to the impending rains, the cultivator harvested the crop before the due date. They had given intimation to the Patwari but the latter did not deliver the message to the Agricultural Assistant in time. In general, the cultivators of all the selected fields willingly agreed to have the experiments done in their fields. They readily arranged for the labour required for harvesting, and, in their turn, were paid a fixed amount towards labour charges. As a rule, wherever possible, the operations of harvesting, threshing, winnowing and weighing were done on the same day, but in cases where the produce was moist, it was allowed to dry up under the care of the Assistant and Kamdar and was threshed after a day or two in their presence. The produce obtained was invariably weighed with the help of standard weights supplied to the staff.

12. The work was initiated and inspected by one of us by continuous touring during the working period in two rounds. First-hand information about the difficulties of the workers was gathered during these visits; for example, almost all the workers pointed out the risk of attacks by poisonous snakes which they had to take in walking on the bunds and through the tall grass. Then again, difficulty was experienced in measuring the dimensions of the field. As per instructions, the assistants were asked to measure the length and breadth of the field in steps. But this was often not practicable owing to obstructions, *e.g.*, thickets, trees, and hedges. A

minor departure was therefore found necessary in the measurement of length and breadth of the selected fields. These were either measured on the maps or in the field by bamboo sticks and then converted into steps.

13. The returns which numbered about 500, were scrutinised in the Statistical Section at New Delhi as they came in. The scrutiny showed that the information supplied including that on the general conditions of the crop at the time of the selection and on the changes in the condition of the crop since the date of selection was detailed and complete.

14. The season was somewhat abnormal from the point of view of the rice crop. The rainfall was deficient, and there was little of it between the 10th September and the 26th October. This being the most vital period of grain development, the mid-late and late varieties suffered throughout the district. The loss was considerable in the case of the crops growing in salt land. In some of the villages in these areas, the crop failed to a large extent. There was heavy rain and storm for about four days from the 27th October onwards, particularly in the Alibagh Taluka, when the late varieties were being harvested. This made the crops lodge in water and consequently some of the ripe grains were shed in the fields and a few grains in the earlies even germinated.

15. Table I shows the results in respect of the average estimated yield of dried paddy (in husk) and of rice (taken at 2/3 the weight of dried paddy)

TABLE I

Name of taluk	Acreage under paddy	Average yield of dried paddy in lbs. per acre	Average yield of rice in lbs. per acre	% of Standard Error of the estimates of Columns 3 & 4	Total out-turn of rice in tons
Karjat	39350	1843·99	1229·39 (36)	10·84	21596
Mahad	36359	1540·08	1026·77 (37)	5·61	16666
Panwel	63055	1789·07	1192·77 (26)	9·64	33576
Pen	36023	1318·98	879·36 (36)	9·30	14142
Mangaon ..	34275	1524·29	1016·24 (33)	7·90	15549
Roha	27196	1940·97	1294·04 (27)	4·64	15711
Alibag	39211	1338·02	892·06 (30)	15·78	15615
Whole district ..	275469	1620·39	1080·32 (223)	3·36	132855

Figures in the parenthesis indicate the number of plots on which the estimate is based.

for each taluk and for the district as a whole together with sampling errors. The last row of the table shows these figures for the district as a whole. The district average is a weighted average of the taluk mean yields, weights being in proportion to the area under paddy. The method of calculating the standard error of the taluk and the district estimates of mean yield is explained later on. It will be seen that the average yield of rice for the district as a whole is 1080 lbs. per acre as against the official figure of 1043 lbs. per acre. The sampling error of the district estimate of mean yield is less than 3½ per cent.

16. Table II shows the figures for the average yield in lbs. per acre of dried paddy and of rice (taking rice at 2/3 the weight of dried paddy) for

TABLE II

Name of category	Average yield of rice in lbs. per acre	% Standard Error of Column 3
Early	910·71	4·76
Mid-late	870·71	5·15
Late	1345·74	5·71

the early, mid-late and late varieties with the percentage standard errors. It will be seen that the average yield of the early and mid-late varieties is of the same order, but the average estimated yield of the late variety is about 50 per cent. higher than either. These varietal differences are inclusive of all factors such as land, sowing date, etc., which differentiate early, mid-late and late varieties and do not refer only to differences due to seed.

17. The figures for dried paddy reported in Tables I and II do not refer to the weight of paddy which is threshed on the same day as it is harvested, but have been corrected for a number of factors, *e.g.*, driage, area under field boundaries, and losses in the process of harvesting, threshing and winnowing, etc. It is common knowledge that the cultivators allow the harvested produce to dry in the field and/or on the threshing floor for a few days before threshing it. It is consequently necessary to allow for the resulting driage to bring the reported results in line with the actual practice. Equally, the yield per acre derived from the results of crop-cutting experiments relate to the area sown with paddy excluding the area covered by the field boundaries. On the other hand, the official figures of acreage include the area covered by the field boundaries within each survey number. It is therefore clear that we should correct either the acreage or the mean yield by reducing it by the proportion of the area covered by these boundaries. Again, we find

that small losses invariably occur during the process of harvesting, threshing, winnowing and weighing. There is no means other than the reports of the field staff for estimating these losses. The losses due to driage have been estimated from returns received from the field staff. The correction needed on account of the field boundaries has been estimated from a knowledge of the length and breadth of fields and the width of the field boundaries. We have combined the losses due to the acreage covered by field boundaries and the losses in the processes of harvesting, threshing and winnowing with the loss on driage into an over-all correction factor and have applied it for estimating the final yield of dry paddy. The over-all correction factor for the district is 5·5 per cent. It is the figures thus corrected which have been reported in Tables I and II.

18. Table III shows the results of the analysis of variance of plot yields for each of the different taluks and Table IV shows the analysis of variance results for each of the three early, mid-late and late varieties. The analysis of variance pooled over the district is shown at the bottom of each table. The standard error of the district estimate of the average yield was calculated from the analysis of variance table for the district, as the results of the Chi Square test of significance had shown that there was no evidence to suggest that the mean square between villages in the different sub-strata were different.

TABLE III

Name of Taluk	Variance due to	d.f.	m.s.
Karjat	Between varieties (Early, Mid-late, Late)	2	94940·57
	Between villages	9	19574·72
	Within villages	24	10448·72
Mahad	Between varieties do.	2	35304·27
	Between villages	9	8510·89
	Within villages	23	7723·47
Panwel	Between varieties do.	1	182835·99
	Between villages	7	36497·02
	Within villages	17	12995·13
Pen	Between varieties do.	2	22376·73
	Between villages	9	34730·17
	Within villages	24	7350·49
Mangaon	Between varieties do.	2	44839·86
	Between villages	8	17047·75
	Within villages	22	16414·87
Roha	Between varieties do.	1	124064·08
	Between villages	7	9414·90
	Within villages	18	7874·48
Alibag	Between varieties do.	2	250391·05
	Between villages	7	38245·67
	Within villages	20	4101·22
Whole District ..	Between taluks	6	49174·01
	Remainder	12	100217·07
	Between villages	56	23050·49
	Within villages	148	9070·59

TABLE IV

Name of varieties	Variance due to	d.f.	m.s.
Early ...	Between Taluks	4	13421·71
	Between Villages	7	10300·80
	Within Villages	23	13113·06
Mid-late ..	Between Taluks	6	23979·31
	Between Villages	28	17469·24
	Within Villages	69	7266·99
Late ..	Between Taluks	6	42476·61
	Between Villages	21	34742·05
	Within Villages	56	9632·69
Whole District ..	Between Varieties (Early, Mid-late, Late)	2	522613·25
	Remainder	16	28276·41
	Between Villages	56	23050·49
	Within Villages	148	9070·59

19. When the number of fields sampled from each village is the same, then the sampling error of the taluk and the district estimate of mean yield are directly obtained from the mean square between villages in the Analysis of Variance Table. Usually, on account of some reason or other, the number of fields is unequal. In the present case, there is one village in Mahad and one in Panwel taluk where only two experiments were conducted instead of three. In such a case, as pointed out by Cochran,[4] the sampling variance of the taluk mean yield is given by:

$$\frac{V \Sigma n_i^2}{N^2} + \frac{F}{N'}$$

where V and F denote the estimates of the true variance between villages and between fields within villages respectively, and are obtained by equating the mean squares between villages and between fields within villages in the analysis of variance with

$$\frac{V}{m-1}\left\{N - \frac{\Sigma n_i^2}{N}\right\} + F \text{ and } F,$$

where n_i = number of fields in the ith village,

N = number of fields in the taluk,

and m = number of villages in the taluk.

When the number of fields in each village is the same, then the formula for the sampling variance of the taluk mean yield reduces to $\frac{V}{m} + \frac{F}{mn}$ which is identical with the mean square between villages in the analysis of variance table. The sampling variance of the estimate of mean yield for the district

was calculated from the formula. $\frac{V}{\Sigma m}+\frac{F}{n\Sigma m}$ where V and F refer to the estimates of the variance for the district.

20. Turning to Table III, it will be seen that in all the taluks the mean square between villages is larger than the mean square between fields within villages and in Panwel, Pen and Alibag the difference is significant. Table IV also shows that the variance between villages is significantly larger than the variance between fields within villages in the case of the mid-late and late paddy, but the difference is negative and non-significant in the case of early paddy. The district analysis shows that the mean square between villages is more than twice the mean square between fields within villages, and that varieties represent a predominant source of variation. These results concerning the relative magnitude of the variability between and within villages have an important bearing in determining the number of experiments and their distribution as between villages and fields in a village for estimating the mean yield with a given accuracy. The formula for sampling variance of mean yield shows that for a given number of experiments, the estimate of mean yield attains the maximum precision when the number of experiments is so distributed that one experiment each is conducted in a different village of the stratum and that the precision of the estimated mean yield falls off when the number of experiments per village is increased at the expense of the number of villages in the district. This will be clear from Table V showing the number of villages that must be sampled for a given

TABLE V

No. of fields per village	No. of villages required for different % of Standard Errors		
	5%	7·5%	10%
1	47	21	12
2	32	14	8
3	26	12	7
4	24	11	6
6	21	9	5
8	20	9	5

number of fields per village and for one plot per field in order to estimate the district mean yield with a given accuracy. It will be seen that there is considerable advantage in increasing the number of fields from one to two, but the advantage is rapidly lost with further increase in the number of fields. Thus, whereas the number of villages required for obtaining an estimate with five per cent. error decreases by 15 when the number of fields is increased

from one to two per village, the number of villages required decreases by only 6 when the number of fields is increased from two to three. There is hardly any material difference in the number of villages that must be selected when the number of fields is increased from three to four and more. The number of villages shown in the table refers to the whole of the district and is to be distributed among the taluks in proportion to the area under paddy. It is of course subject to sampling error since the values of the true variance between villages and within villages are themselves only sample estimates of the population values.

21. The calculations in Table V have been based on the assumption that it is possible to eliminate the differences in yield between the early, mid-late and late paddy from the error of the final estimate of yield by employing varieties as substrata. This may not be always possible, since the areas under varieties are not known for all districts. In the present scheme special effort was made through the Revenue Department to obtain these statistics. Ordinarily, therefore, independent samples of villages for early, mid-late and late varieties cannot be selected in the absence of area statistics under individual varieties. The requisite number of fields in the selected villages will have to be chosen irrespective of whether the fields grow early, mid-late or late paddy. As the mean square due to varietal differences in the Analysis of Variance is significantly larger than the mean square between villages, an unrestricted selection of fields in the selected villages will lead to the increase in the value of the mean square between fields within villages and also in the value of mean square between villages itself. It is difficult to form any precise idea of the extent by which the variance of the estimated mean yield will be increased on this score. One would be on the safer side in summing up the sums of squares due to varieties and due to between villages and dividing this sum by the sum of the degrees of freedom in order to form an idea of the resulting value of the mean square between villages. On this basis it would seem that the number of villages will have to be increased by over two-thirds in order to estimate the district mean with the same precision as if each taluk was stratified by early, mid-late and late varieties.

22. As there is a difference of about a fortnight between the maturing stages of the early, mid-late and late varieties, it may be necessary to visit the same village more than once in the case of unrestricted selection, since the fields mature at different times. A method which enables us to harvest the selected fields in a small number of visits such as stratification, by early, mid-late and late varieties is not only statistically superior, but is economic and convenient. It, however, appears that we may have to

forego this advantage in the absence of area statistics under early, mid-late and late paddy. The early variety by itself occupies less than 5 per cent. of the total area under paddy; moreover, the average yields of the early and mid-late paddy are of the same order. Even if, therefore, acreage under early and mid-late is combined and area figures are known separately for only two crops, early plus mid-late and late paddy, it will still be a great advantage to stratify taluks by these two categories. In the absence of separate area statistics for early plus mid-late and late paddy, a larger number of villages will have to be selected, and a field worker may be required to visit a village on an average more than once. Thus, whereas only four villages per taluk are required with stratification, seven villages per taluk would be required without it. The charge of 7 villages per man may prove too large for him to visit all the villages in his jurisdiction within the period available for harvesting. Indeed, additional staff may be required. With four villages per taluk, two from the early plus mid-late paddy, and two from the late paddy, work can be more easily managed by the existing departmental staff in the course of their normal duties. It would thus seem that stratification by varieties makes an appreciable difference to the plan of work and that it would, therefore, be desirable to ascertain separately the area under late paddy. Until the time steps are taken to ascertain the area under late paddy in each district, we shall have to be content with a slightly lower degree of precision for the district estimates of average yield, assuming, of course, that the work has to be carried out by the existing departmental agency.

23. It was not a part of this scheme to determine the optimum size of plot. This has been investigated elsewhere. A brief description of the results of this investigation is given in Table III. The conclusion reached is that both on theoretical and practical grounds a large-size plot is to be preferred to a small size one such as is marked by a rigid or semi-rigid frame. Within the range of large-size plots, however, it may be feasible to adopt a plot of smaller dimensions consistent with the requirement that errors due to the measurement of the plot and the processing of the produce do not contribute more than a negligible proportion of the produce harvested. All the available information suggests that there is a definite risk of obtaining a biased yield estimate under Indian conditions by conducting crop-cutting experiments with rigid or semi-rigid frames of area smaller than 20 sq. ft. Crop-cutting experiments on paddy in the Bombay Province are usually conducted on one-guntha plots of size 33′ × 33′. There need be no objection to the use of this size to which the officials are accustomed since the results show that the choice of one plot in preference to another within the range

of 1/80th to 1/40th of an acre is of little importance in improving the precision of the estimate of yield. One has further to ensure that fields which are too small to contain a plot do not form more than a negligible proportion of the total population. The results of this survey show that the proportion of fields rejected on this score is less than 2%, but there are approximately four per cent. of fields whose breadth is between $16\frac{1}{2}$ and 33 feet all of which will have to be rejected in case the official plot size is adopted. It is accordingly suggested that the plot size $33' \times 16\frac{1}{2}'$ may be adopted in the hilly districts of Konkanpatti, but elsewhere in the province the present official size $3 3' \times 33'$ may be retained.

24. There is an inherent difficulty in sampling from a finite field. Whatever the size of plot, the central portion of the field is relatively over-sampled when compared with the surrounding area. Consequently there is a likelihood of obtaining a biased estimate of the mean yield if the yield of plots located near the borders is different from that of plots in the remaining part of the field. The whole question has been considered at length elsewhere. It has been shown that the bias, if it exists, can be corrected by introducing a correction factor such that it will equalise the chance of every portion of the field being included in the sample. An application of this correction factor to plot yields in the present case shows that the estimate of average yield remains unaltered, the difference between the original estimate and the corrected one being less than one per cent. The point was also examined by tabulating the frequency distribution of plot yields according to the relative position of the plots in the field. The results are shown in Table VI. The relative position of the plot defined by the quotient of the random number of the starting point of the plot by the length of the field

TABLE VI

Length			Breadth		
Relative position of plots	No. of plots	Average yield per plot in chattacks	Relative position of plots	No. of plots	Average yield per plot in chattacks
0·0 to and including ·1	19	266·94	·0 to and including ·1	26	300·53
·1 – ·2	16	395·81	·1 – ·2	24	306·62
·2 – ·3	27	365·03	·2 – ·3	21	361·23
·3 – ·4	30	336·63	·3 – ·4	16	333·25
·4 – ·5	30	318·46	·4 – ·5	25	319·64
·5 – ·6	18	312·27	·5 – ·6	25	318·12
·6 – ·7	17	360·41	·6 – ·7	22	330·31
·7 – ·8	27	298·77	·7 – ·8	21	377·76
·8 – ·9	19	392·68	·8 – ·9	25	335·56
·9 – 1·00	19	342·75	·9 – 1·00	17	368·17

less the length of the plot is given in column 1. The number of plots having the relative positions defined in column 1 is shown in column 2, and the average yield per acre is given in column 3. Columns 4, 5 and 6 give the same figures when the relative position is defined along the breadth of the field. The Chi Square Test of significance for the number of plots in the different class intervals shows that the location of the starting point of the plot was genuinely random, as was to be expected. A comparison of the variation within classes with the variation between classes shows that the estimate of the yield per acre is not influenced by the relative position of the plot in the field.

25. The question of the shape of plot is being investigated in other provinces. It appears, however, that shape has no influence on the precision of the mean yield. There is, however, an advantage in marking out a triangular plot in place of a rectangular one, and that is, that the time taken for marking out the former is much less than the time required for marking out the latter. A suitable triangular plot whose size is approximately half a guntha will be an equilateral triangular plot with sides 33′. The only disadvantage in the adoption of this plot size will be that on account of its breadth of 28′, it will lead to a slightly larger rejection of fields than a plot size of 33′ × 16½′.

26. Random sampling implies equal chance for every plot in a taluk to be included in the sample. This can be done by considering the whole of the area of the taluk divided into plots and selecting random sample from these plots. This, however, is not a feasible method. The practicable method of selecting a random sample of plots is to select a random sample of fields and locating sample plots in each of these selected fields. The easiest way of selecting a random sample of fields is to select random groups of fields and then to select fields at random from each of these groups. The best group is the one for which the list of fields is readily available and in which the fields are readily located with the help of the local officials. The investigations of Panse and Kalamkar[5] in India show that there is no difficulty in selecting villages at random, nor is there any difficulty in selecting fields at random in the selected villages. In this method, however, every unit area does not get an equal chance of selection. Smaller villages and smaller fields get relatively larger chance of selection. This requires correction particularly if there is an association between the area and the yield. One method of correcting is by forming progressive totals of areas and of fields in selected villages before a selection is made. Another method would be to work out weighted averages by weighting the yield of each plot with the

area of the corresponding plot and weighting the average yield of a village with the corresponding area under paddy. These weighted averages have been calculated and show that these sources of bias in sampling have no effect on the estimated yield. It appears that no correction would be necessary in practice.

27. To conclude, it will be seen that it is practicable to use the method of random sampling for estimating the district mean yield with reasonable accuracy. In particular, the results show that by sampling about 45 villages in a district, distributed in proportion to the area under paddy in the different taluks, and three fields in each selected village, the average yield of the district can be estimated with a sampling error approaching five per cent. The amount of sampling required is considerably less if sampling is done separately for early, mid-late and late varieties, but this requires a knowledge of the area figures under these varieties.

Experience shows that the charge of 45 villages is too large for the existing staff of the Department of Agriculture, for the staff consists on the average of only one Agricultural Assistant per taluk. If, however, experiments are distributed between the staff of the Departments of Agriculture and Revenue, as appears to be the practice at present, and further the work is so distributed that independent samples from the same area are assigned to the staff of the two Departments, then, not only will it be possible to manage the work in the limited time available for harvesting, but we shall also have two independent estimates of the yield per acre for each district. These data might well form the basis for ascertaining the care with which the field work is done. If an agricultural agency is not available, the only alternative available is to entrust the work to the staff of the Department of Revenue in a way that it will fit into the existing machinery of the Department. Whether the work is distributed between the Departments of Agriculture and Revenue, or is exclusively carried out by the staff of the Revenue Department, the field work will have to be so organised that it will fit in with the administrative arrangements of the department by assigning the field work within each Circle to the respective local Revenue staff.

Under the existing official procedure, the yield per acre is estimated by multiplying the normal yield by the condition factor. The normal yields are, however, based largely on considerations of general experience, and as such are purely arbitrary. There is no means of knowing the extent to which they are biased. The condition factor is a subjective estimate of the crop in terms of the normal, and is subject to unknown errors. The advantage of estimating the yield per acre by the method of random sampling is that the subjective

element is altogether eliminated from the process, and what is even more important, the precision of the estimated yield is known. The work will naturally require strong statistical direction, for planning the surveys, for analysing the results and for incorporating the results in successive year's plans and also for guarding against the possibility of the random sampling method degenerating into inefficient routine by ensuring adequate supervision of the field work. It is gratifying to note that the Government of Bombay have appointed a Statistician to carry out the work on the lines indicated in this paper for estimating the yield not only of paddy but of all crops in the provinces.

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ON *LECUDINA PELLUCIDA* (KÖLLIKER) MINGAZZINI (1891) FROM THE GUT OF *NEREIS CHILKAENSIS* SOUTHERN

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Introduction

While examining the polychætes from the Madras Harbour in search of Sporozoan parasites, I frequently came across a gregarine infecting the gut of *Nereis chilkœnsis*. A perusal of the existing literature soon convinced me that all the previous accounts of gregarines from species of *Nereis* are fragmentary and that there is great diversity of opinion especially regarding the nature of the epimerite, if we are to take for granted that all these accounts refer to one and the same species. Apart from a brief note by Leger (1893), nothing seems to be known about its life-history.

Kölliker as early as in 1848 recorded and figured a gregarine from *Nereis* (unknown species) and called if *Gregarina pellucida*. Lankester (1863) considered *Gregarina pellucida* as a monocystid and described it as *Monocystis nereidis* (pellucida Köll.). Mangazzini (1891) observed the same form and called it *Lecudina pellucida*. Two years later Leger (1893), working independently, created the new genus *Doliocystis* for the species described by Mingazzini. He described two forms, *D. nereidis*, from the intestine of *Nereis cultrifera* (Gr.) and *D. polydoræ*, from the gut of *Polydora agassizi*

Clap. The earlier work of Mingazzini was overlooked by subsequent workers and the name used by Leger came into more frequent use. Labbé (1899) raised the genus to the status of a family, the Doliocystidæ. Brasil (1904, 1909) from an examination of Léger's type species and of others came to the conclusion that *Doliocystis nereidis* is apparently synonymous with *Doliocystis pellucida* (Köll.) and found these parasites living in the gut of *Nereis* (*Lipephile*) *cultrifera* (Gr.). Kamm (1922) considers that both *Ophiodina* Mingazzini and *Doliocystis* Léger are synonymous with *Lecudina* Mingazzini. She created the family Lecudinidæ and defined it as including 'Non-septate Gregarines inhabiting the digestive tract of polychætes Epimerite a simple knob'. Reichenow (1929) amplified and amended. Kamm's definition as follows: 'Body non-septate, distinguishable from the Monocystids by the protoplasm in the anterior portion possessing finer granules. A typical epimerite for attachment to the wall of the gut of the host is present, but may be lost in fully grown individuals. Syzygy does not occur. Sporocysts oval, with a thickening at one pole. Parasites of marine annelids'.

The gregarine in *Nereis chilkænsis* undoubtedly agrees with the genus *Lecudina* in all its features. As already stated there has been want of agreement in the descriptions of gregarines from species of *Nereis*, even though there are reasons to believe that all the authors apparently refer to one and the same species of parasite, which, under the present accepted system of classification, is referred to as the Genus and type species of the family Lecudinidæ Kamm, and named *Lecudina pellucida* (Köll.) Mingazzini. I have not examined *Nereis cultrifera* and, as such, in drawing any comparison of the present form with the Gregarine recorded from the above host, I have to depend entirely on the meagre descriptions and figures given by previous workers. The epimerite of the gregarine from *Nereis chilkænsis* is very much like that of *Doliocystis pellucida* (Köll.) Ming. as described and figured by Brasil (1904). The shape of the trophozoite is, however, different and resembles the figure given by Kölliker. Kolliker evidently did not observe the epimerite, but both this author and Mingazzini mention the retractility of the anterior end within the body and in addition they show the protoplasm at the anterior end as nearly transparent and different from the rest of the body. Léger (1893) describes the epimerite in *Doliocystis nereidis* as intracellular and caducous, but Brasi (1904, 1909) who restudied Léger's type species has shown that this is an error, the epimerite being a permanent invaginable sucker-like organ.

Taking for granted that both these authors refer to one and the same parasite, my observations on the gregarine from *Nereis chilkænsis*, confirm

Brasil's description as regards the nature and structure of the epimerite. I am, therefore, reluctant to create a new species for the form seen by me but instead, propose to retain it as the Genus and type species of the family Lecudinidæ Kamm. The general outline of life-history agrees with the brief description given by Léger for ***D. nereidis***, but the spore formation takes place outside the host in sea-water as in ***Lecudina brasili*** (Ganapati and Aiyar, 1937). It is the purpose of the present paper to give in detail the morphology and life-history of what I consider to be ***Lecudina pellucida*** (Köll.) Ming. with a view to filling the gaps left by previous observers, and to compare the form with other known species of the genus. In this work I have been fortunate in being able to examine a large quantity of material both living and fixed instead of being content with a few cysts collected at random and with great difficulty as was the experience of earlier authors.

Material and Methods

All the worms examined were collected from the Madras Harbour, where they occur in fairly large numbers in the crevices of stones or creeping on shells of oysters. As soon as they were brought to the Laboratory they were thoroughly cleaned and kept in shallow glass troughs in sea water where they remained healthy for about a fortnight with daily change of water. No worm examined was completely free from the parasite, the nature of infection being only a question of degree. Owing to the large size of the worm and the thickness of its body-wall, it was necessary to cut the worm into bits and to examine the fluid oozing out from the alimentary canal, before deciding whether there are parasites or not. Fresh observations, smears and sections were made adopting the methods described in a previous paper (Ganapati and Aiyar, 1937). Cyst formation takes place in the mid-gut and the cysts are passed out along with the fæcal pellets of the worm. After clearing the debris they were immediately fixed in Bouin-Duboscq's fluid kept at 60° C. in the bath for one hour and at room temperature for 24 hours. Embedding was done in paraffin and sections made 4 to 8 microns in thickness. Heidenhain's hæmatoxylin was used for staining both sections and smears.

The living parasite was observed in a medium of the body-fluid of the host diluted with sea-water. Cysts kept in petri-dishes in a moist chamber completed their development in about 3 days, liberating the spores.

Observations on the Gregarine

The earlier stages of the parasite are intracellular within the epithelial cells of the gut of the host (Photomicrograph 1). They are mostly found in the middle region of the intestine where the epithelium is thick. The

youngest parasite was ovoidal and measured 8 microns along the longer axis (Fig. 1). The cytoplasm is uniformly granular and the pellicle thin. The nucleus which is more or less centrally placed is spherical with a distinct nuclear membrane. There is usually one and in some cases two deeply staining karyosomes closely applied to the nuclear membrane. When there are two, one is considerably larger than the other. The nuclear sap is clear without any chromatin. In sections the parasite appears surrounded by a vacuole within the protoplasm of the host cell. It appears possible that the vacuole is formed at the time of fixation and that in the living condition this space is occupied by a clear fluid which forms the nutriment for the growing parasite and which is absorbed through the thin pellicle covering the parasite.

As growth proceeds one end of the parasite facing away from the gut lumen gets differentiated from the rest of the body. This end which we may hereafter call the anterior end is in the form of a blunt rounded knob (Fig. 2). The cytoplasm at this end is finely granulated and clearly marked out from the general body protoplasm which is coarse consisting of larger granules. The changes mentioned above are visible in parasites 14 microns by 10 microns. At a still later stage when the parasite has attained a length of about 20 microns, it is found attached to the host cell by a short truncated cone-like structure at the anterior end (Fig. 3). This organ of attachment or epimerite persists even after the parasite outgrows the host cell and hangs down into the gut. From observations made on the adult gregarine it is gathered that the attachment is effected by a sucker-like action of the cup-shaped end of the epimerite. It is probable that in addition to being an organ of attachment the epimerite may also function as an organ through which nourishment is absorbed from the host's tissue.

The host cells containing the earlier stages of the parasite are not much differentiated from the surrounding cells. With the growth of the parasite, however, the infected cells undergo hypertrophy, the protoplasm becomes more vacuolar and the nuclei which at first show signs of hypertrophy later on completely degenerate and get pushed to one side. When a large number of parasites are concentrated the epithelium in the region is swollen and projects into the gut lumen (Photomicrograph 1). When the cytozoic phase is over, the host cell is ruptured and the animal hangs down into the lumen of the gut still attached to the epithelium by the epimerite (Fig. 4).

The Trophozoite

The adults or trophozoites occur in large numbers in the middle and posterior region of the intestine (Photomicrograph 2). They are rarely seen in the anterior region. To observe the living parasites the worm was

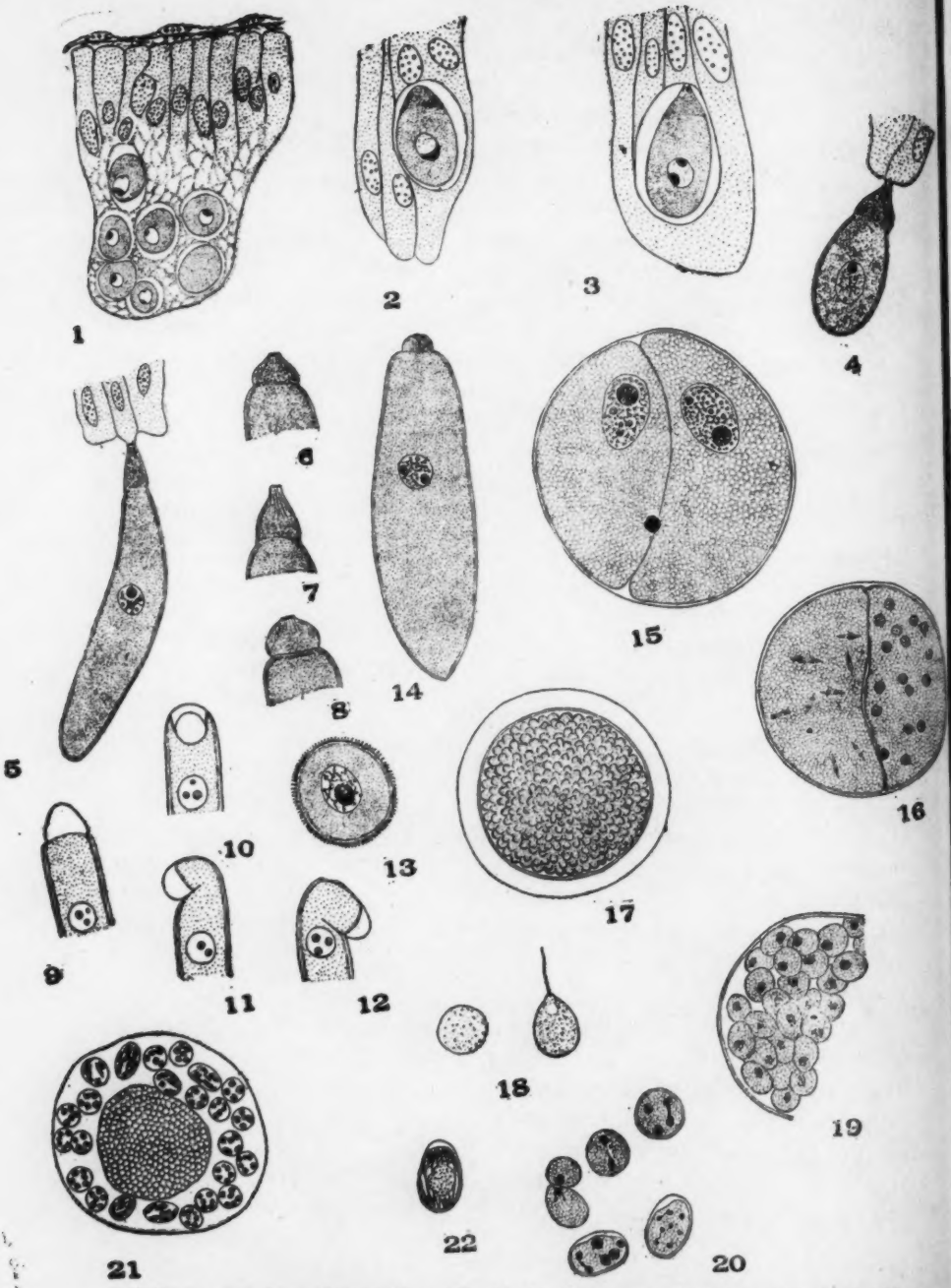
cut into small bits and teased out on a slide with a fine pair of needles. The body fluid of the worm mixed with a drop of sea water served as a very favourable medium for observing the fresh parasites, for a considerable length of time, without any apparent degeneration.

The free trophozoites are elongated and cylindrical with a thick pellicle covering the body (Fig. 5). The cytoplasm is uniformly granular except for a small zone at one end which is transparent and hyaline, clearly marked out from the general body protoplasm. In some instances a clear line or septum separating these two regions is visible but, as explained in a later part of the paper, a true septum is not present, the deceptive appearance being brought about by the sinking in of the anterior region or protomerite into the main body. These two regions, on comparison with the Septate or Cephaline Gregarine, may be called the protomerite and deutomerite respectively. The epimerite is hardly visible in fresh preparations, being almost always in a retracted condition in the free parasite.

The differentiation of the protoplasm at the anterior end from that of the remainder of the body was illustrated both by Kölliker (1848) and Mingazzini (1891). Léger (1893) does not seem to have noted this difference in *Doliocystis nereidis*. Brasil (1904, 1909) clearly brings out this difference in *D. pellucida, D. elongata* and *D. legeri*. I have drawn attention to this difference in *Lecudina brasili* (Ganapati and Aiyar, 1937) and have since found the same feature in two other new forms of *Lecudina* (Ganapati, 1946).

The free trophozoites perform active gliding movements without any change in body shape. The change in shape is mostly confined to the protomerite and a small region of the deutomerite behind it (Figs. 9 to 12). This part of the parasite bends over till the two limbs are nearly parallel and then the animal suddenly straightens itself with a jerk. There is another type of movement in which the protomerite alone takes part. The protomerite is capable of contraction and elongation in an antero-posterior axis and, in addition, it performs a twisting movement from side to side. When the parasite glides along it can be noticed that the protomerite sways from side to side.

The trophozoites attached to the gut epithelium grow till they reach a size of about 100 to 150 microns in length and 20 to 30 microns in width. The body is elongated and cylindrical as seen from sections (Fig. 13). The posterior end is bluntly rounded while, anteriorly, the deutomerite is imperceptibly continued into the protomerite and the epimerite when the animal is in a fully extended condition (Photomicrograph 3). However, as usually seen, there is a waist-like constriction or neck between the protomerite and



All figures were drawn from sections with the aid of a camera lucida at stage level, with Zeiss apochromatic oil immersion objective and compensating oculars. Unless otherwise stated,

the material was fixed in alcoholic Bouin-Duboscq and sections stained in Heidenhain's iron-alum hæmatoxylin. The magnifications given are those at which the drawings were made but Figs. 1–22 have been reduced to half.

Fig. 1. Part of gut showing a few intracellular parasites × 720.
Fig. 2. A slightly advanced intracellular stage in which the protoplasm is differentiated at the anterior end × 1080.
Fig. 3. A still later stage where the epimerite is present × 1080.
Fig. 4. The trophozoite just liberated from the host cell and attached to the gut epithelium × 1080.
Fig. 5. A full-grown trophozoite × 444.
Figs. 6 to 8. The anterior end of the parasite showing different stages in the retraction of the epimerite × 1080.
Figs. 9 to 12. Freehand sketches of living parasites showing the changes of shape assumed by the anterior end. ×
Fig. 13. A cross-section of the trophozoite in the region of the nucleus × 1080.
Fig. 14. A ripe sporont ready for association × 444.
Fig. 15. Cross-section of a cyst × 1080.
Fig. 16. Section of a later cyst × 1080.
Fig. 17. A fresh gametocyst × 480.
Fig. 18. The gametes male and female × 1440.
Fig. 19. Part of section of a gametocyst showing fully formed gametes × 1080.
Fig. 20. Stages in the formation of the sporoblast and sporocyst × 1440.
Fig. 21. Section through a ripe cyst showing sporocysts and crystal residuum × 1080.
Fig. 22. A ripe sporocyst showing sporozoites and sporocyst residuum × 1440.

the main body segment. The nucleus is situated in the anterior half of the deutomerite in the middle of the body. The nucleus is spherical measuring about 10 microns in diameter. There is a distinct nuclear membrane and the nuclear sap is filled with a thick network of chromatin granules. There are 3 to 5 karyosomes of which one is always much larger than the others.

The cytoplasm is coarsely granular in the main body segment and stands in sharp contrast with the finely granulated cytoplasm of the protomerite. The pellicle is thick and is traversed by longitudinal ridges. In transverse sections the trophozoites are circular and the ridges separated by shallow grooves are clearly visible (Fig. 13).

The protomerite is marked out from the deutomerite not only by the difference in the consistency of the cytoplasm in these two regions but also by the capacity of the former to assume different shapes according to its degree of extension or contraction. This difference is markedly evident in living parasites. There is, however, no septum between the two regions as in the Cephaline Gregarines. Often a false septum is seen in fresh parasites brought about by the sinking in of the protomerite into the main body segment. Figs. 6 to 8 explain how this is brought about.

The epimerite is a short conical structure continued directly from the anterior end of the protomerite. The tip of the epimerite by which attachment is effected is cup-shaped and adhesion to the host's tissue is effected by a sucker-like action of this end. The rim of the epimeritic cup stains dark with hæmatoxylin. The whole epimerite is capable of being retracted into the protomerite and in such a condition the parasite appears attached by the protomerite. The pellicle over the main body is fairly thick, but it becomes thin over the protomerite and epimerite. The pellicle in the protomerite and epimerite is traversed by a number of longitudinal striations which appear to be connected with the special contractility of this region.

Both Mingazzini and Kölliker mention the retractility of the anterior end within the body in the forms they studied. Léger, however, describes the epimerite in *Doliocystis nereidis* as 'le segment intracellulaire' which is in the form of a 'simple bouton'. He further states that the epimerite is caducous and that it is left behind when the animal breaks away before attaining the free state. Brasil (1909) who restudied Léger's type species came to the conclusion that the epimerite is not caducous but that it is 'un appareil permanent, invaginable, de la meme categorie que ceaux de *Doliocystis elongata* on de *Lankesteria ascidiæ*'. My observations on the present form has amply substantiated Brasil's findings regarding the nature of the epimerite.

The full-grown trophozoites ripe for association and encystment detach themselves from the gut wall and lie free in the lumen. These mature forms or sporonts differ from the trophozoites in their greater girth as well as the denser consistency of the cytoplasm. The protomerite does not appear to share this increase of girth and it is seen as a blunt knob-like structure at the anterior end (Fig. 14).

Association and Cyst Formation

The ripe sporonts come together in pairs and get attached to each other by their anterior ends. The epimerite serves to hold them together till association is completed. The parasites so attached shorten in length with a proportionate increase in girth. The association becomes lateral. In the next stage they slowly begin to rotate, both together or independently, either in the same direction or in opposite directions. This process goes on for about three quarters of an hour by which time a definite cyst wall, secreted by the activity of the parasites, becomes visible. The cyst formation is completed in about 3 hours. The completed cysts are passed out along with the fæcal pellets of the worm. The cysts were collected at regular intervals

and, after cleaning, fixed for sectioning. It was possible to obtain in this manner cysts showing different stages of gametogenesis and spore formation. When kept in clean sea water or in a moist chamber the cysts completed their development in about 3 days liberating the ripe spores.

A few minutes after the cysts are passed to the outside a thick gelatinous covering makes its appearance around each cyst. This covering or ectocyst is transparent and seems to be formed by the swelling up of a substance on the surface of the cyst wall. The formation of the ectocyst seems to ensure protection against bacteria and other micro-organisms which try to attack the cysts, in their new environment.

The cysts are spherical and ordinarily measures 30 to 40 microns in diameter (Fig. 15). In the fresh condition the cytoplasm of the gametocytes is dense and the partition wall separating them clearly visible.

Gametogenesis and Sporogony

The details of the development of the cyst until the spores are formed can be gathered only from a study of sections. Examination of sections of cysts fixed 2 to 3 hours after they are passed to the outside showed the following changes (Photomicrograph 4). The nucleus has increased in size and the chromatin forms a dense cloud filling the nuclear sap (Fig. 15). The karyosomes have also increased in number up to about 10 and they stain a deep mauve, while the chromatin itself remains bright blue. In the next stage the chromatin forms a highly tangled network or spireme. I have not been able to see completion of the first division. Gametocytes containing four or more nuclei were fairly common and the nuclear divisions take place mitotically. The details of the division are very much the same as those described in *Lecudina brasili* (Ganapati and Aiyar, 1937). Fig. 16 shows an advanced cyst containing resting nuclei in one gametocyte and dividing nuclei in the other. A definite spindle is present but the achromatic figure consists of only a centriole without an aster. When the nuclear divisions are complete, they are seen scattered all over the cytoplasm. Each nucleus is in the form of a few globules of chromatin connected together by a thin network. Just prior to gamete formation there is further condensation of chromatin and the cytoplasm presents a furrowed appearance. The cytoplasm ultimately breaks up into as many bodies as there are nuclei leaving a large spherical mass in the centre, which plays an important role in the dehiscence of the cyst and scattering of spores. Gamete formation is completed in about 48 hours and a fresh cyst examined at this stage presents the appearance shown in Fig. 17.

The fully formed gametes may be liberated by applying gentle pressure on the cover-glass and examined in a medium of sea water. Two types of gametes, presumably produced by the two different gametocytes, are seen. One is more or less circular and inert and the other pear-shaped and motile (Fig. 18). On careful examination, after cutting off most of the light under the microscope, a long slender flagellum performing active lashing movements can be seen, attached to one end of the gamete, which is motile. The movement is forwards in the direction of the flagellum. In sections and smears it is very difficult to make out the flagellum. From a comparison with the gametes in other gregarines it is apparent that the motile type represents the male and the non-motile one the female. Both types contain a few refractile globules in the cytoplasm, which are presumably food material made use of in the formation of the spores. In sections each gamete has a closely alveolated cytoplasm and a nucleus made up of a few large globules of chromatin connected together with a network of the same material (Fig. 19).

Copulation takes place between the two anisogamous gametes and the cytoplasmic fusion precedes nuclear fusion (Fig. 20). The Zygote nucleus divides successively twice forming eight nuclei. The sporoblast membrane which has formed meanwhile becomes thick. In the 8-nucleate stage the cytoplasm inside the sporoblast undergoes longitudinal cleavage resulting in the 8 sickle-shaped sporozoites and the sporoblast membrane becomes the sporocyst wall. As seen from sections the sporozoite nuclei are placed at different levels. A spherical sporocyst residuum is present (Fig. 21).

The sporocysts are ovoidal measuring 9 by 6 microns (Fig. 22). The sporocyst wall presents a characteristic thickening at one pole (Photomicrograph 5). The sporozoites may be liberated by applying gentle pressure on the cover-slip. The living sporozoites exhibit feeble twisting movements. One half of the sporozoite is transparent while the rest of the body is granular. The transparent region represents the nucleus, and in sections the nucleus is seen as an elongated homogeneously staining body.

After spore formation is completed a large quantity of residual protoplasm is left behind in the middle of the cyst. This cystal residuum plays an important part in the dehiscence of the cyst and scattering of the spores by its hygroscopic nature and consequent swelling up by absorption of water through the cyst wall, which by now has become very delicate and weak. I have watched this scattering of spores under the low power of the microscope, the liberated spores appearing as whitish-opaque bead-like bodies.

The Genus Lecudina Mingazzini, 1891

Mingazzini founded the genus ***Lecudina*** in the year 1891. Two years later Léger (1893) working independently on the species described by Mingazzini, created a new genus ***Doliocystis*** Léger. The later authors overlooked Mingazzini's work and the name used by Léger came into more frequent use. Kamm (1922) referred back the genus ***Doliocystis*** Léger as a synonym of ***Lecudina*** Mingazzini and created the family Lecudinidæ. She also considered that ***Ophiodina*** Mingazzini is synonymous with ***Lecudina*** Mingazzini. Kamm (1922) included in the family Lecudinidæ, Gregarines from the intestine of marine annelids having a non-separate body, possessing simple symmetrical epimerites, and producing ovoidal spores presenting an asymmetrical thickening at one pole. A closely allied family the Polyrhabdinidæ was created by Kamm (1922) to include the polycystid (septate) gregarines inhabiting the digestive tract of polychætes and possessing varied epimerites. The family Lecudinidæ Kamm included only a single genus ***Lecudina*** and under the closely allied family Polyrhabdinidæ was placed three genera, ***Polyrhabdina*** Mingazzini 1891, ***Sycia*** Léger 1892, and ***Ulivina*** Mingazzini 1891. Kamm states that because of the presence of a septum in the Polyrhabdinidæ 'they must be included in the sub-order Cephalina, but that they stand near the borderline with the Acephalina because of their presence only in polychætes'. Reichenow (1929) considers that the presence of a septum in ***Polyrhabdina*** as described by Kamm is an error, and that the septate appearance is due to the protoplasm in the anterior end of the body being more finely granular. He consequently amalgamated the family Polyrhabdinidæ with Lecudinidæ, and later Reichenow (1932, 1935) included three more genera, ***Ancora*** Labbe, ***Hentschelia*** Mackinnon and Ray, ***Lecythion*** Mackinnon and Ray, in the family Lecudinidæ Kamm as emended by him. Reichenow considers ***Sycia inopinata*** Léger as identical with ***Ulivina elliptica*** Mingazzini. Bhatia (1938) is of opinion that ***Ulivina*** and ***Sycia*** are distinct as all the three known species of ***Ulivina*** namely, ***U. elliptica*** Mingazzini, ***U. rhyncoboli*** (Crawley) Kamm, and ***U. eunicæ*** Bhatia and Setna do not show an important generic character of ***Sycia***, as given by Léger for the type species, namely a knobbed epimerite bordered at the base by a thick ring. I have described a form (Ganapati, 1946) which undoubtedly belongs to the genus ***Sycia*** as defined by Leger and possessing a typical epimerite characteristic of the genus. This form does not show a septum and I am inclined to consider that Léger's confusion probably arose from the fact that the body protoplasm at the anterior end is differentiated from the rest of the body. In this respect ***Sycia*** is as much a dicystid as ***Lecudina*** and ***Polyrhabdina***. It should also be pointed out that Mackinnon and Ray (1931)

Table showing the known species of Lecudina *Mingazzini,* 1891

Name of parasite	Name of Host	Shape of Trophozite	Nature of Epimerite	Measurement in microns	Cysts and Spores	Remarks
1 *L. pellucida* ..	*Nereis cultifera*	Ellipsoidal or bottle-shaped. Protoplasm differentiated at anterior end	Deformable truncated cone ending in a sucker-like cup —Author.	Not given	Cysts small, spores ovoidal. 7 × 5 microns, with thickening at one pole	Triet, Naples and Gulf of Marseilles
Type Species ..	*N. beaucourdrayi*	Protoplasm differentiated at anterior end				
2 *L. leuckarti* ..	*Sagitta* Sp.	Similar to Type Species	..	..	..	..
3 *L. aphroditæ* ..	*Aphrodite aculeata*	..	Long slender 'proboscis'	½" in length	..	..
4 *L. elongata* ..	*Lumbriconereis impatiens*	Elongate-cylindrical	"Piccolo bottone sferico"	500 × 40	..	Intra-cellular stage present
5 *L. heterocephala*	*Nephthys scolopendroides*	Very elongate vermiform. Protoplasm differentiated at anterior end	"A sort of papilla"	..	..	Taken at Naples
6 *L. polydoræ* ..	*Polydora agassizi*	Protoplasm differentiated at anterior end	"D'un tronc de cone a petite base inferieure et il se"	..	..	..
..	*Polydora ciliata*	..				
7 *L.* Sp. ..	*Polymnia nebulosa* *Notomastus exertilis* *Petaloproctus terricole*	..	..	..	..	It is quite possible that several species are involved —Kamm.
8 *L. legeri* ..	*Glycera convoluta*	cylindrical	'A rhizoid fila-	100 × 25	Cysts small, 45	Mediterranean

8 *L. legeri* ..	*Glycera convoluta*	cylindrical	'A rhizoid filament which penetrates to the base of the host cell'	100 × 25	Cysts small, 45 microns in diameter, spores typical	Mediterranean Coast
9 *L.* Sp. ..	*Polydora socialis*	Spindle-shaped	..	..	..	Not enough data to definitely fix the position of the species
10 *L. brasili* ..	*Lumbriconereis* Sp.	Widest in middle and tapering posteriorly	Slendor prolongation from anterior end ending in an anchor plate	150 × 30	Cysts measure 75 in diameter. Spores 6 × 4 with thickening at one pole	Taken at Madras, India
11 *L. eunicæ* ..	*Eunice siciliensis*	Elongate oval widest in the middle narrowing at both ends	A knob-like structure with its cytoplasm differentiated	475 × 94·5	..	Andaman Islands, Port Blair, INDIA
12 *L. lysidicæ* ..	*Lysidice collaris*	Oval, broadly rounded anteriorly and narrower and pounded off posteriorly. No differentiation of protoplasm at anterior end	A long conical trunk	185 × 62·9 370 × 155·4	..	Andaman Islands, Port Blair, INDIA
13 *L. krusadiensis*	*Platynereis abnormis*	Elongated and cylindrical, protoplasm differentiated at the anterior end	Button-like sucker refractile into the anterior end of the body	100–150 × 20–25	..	Earlier stages are intra-epithelial. Taken at Krusadi Island, Gulf of Mannar, INDIA
14 *L. indica* ..	*Lycastis indica*	Elongated cylindrical body tapering at extremities. Protoplasm differentiated at the anterior end	Cup-shaped depression at anterior end functioning as a sucker	100–190 × 30–50	Cysts spherical measuring 40–70 in diameter. Spores ovoidal 12 × 8 microns	Taken at Madras MADRAS
15 *L. longicephala*	*Pisionideus indica*	Elongate, cylindrical, with anterior end drawn out into a long proboscis	Cup-shaped sucker-like mucron	400–631 × 50	..	Taken at Madras, MADRAS

and self (Ganapati, 1946) have not seen a septum in the species of *Polyrhabdina* studied by us.

Kamm (1922) has assigned an intermediate position for the family Lecudinidæ and Polyrhabdinidæ, between the Acephalina and the Cephalina. This, according to her, is justifiable by the non-septate nature of the body and the possession of an epimerite at the same time. The body, in other words, contains only two segments, the main body representing the protomerite and the deutomerite of tricystids (Cephaline) and an epimerite. Minchin (1912) states that ' it is purely a matter of definition whether these forms be considered as Cephalina without a septum, or as Monocystids with an epimerite and adds that though the terms Dicystida and Tricystida (Cephaline) are to be understood in a purely descriptive sense having no taxonomic value on account of some of the dicystid species having had their origin from tricystid forms secondarily by obliteration of their protomerite, ' such forms as the Doliocystidæ appear to be truly and primitively discystid, and are to be regarded as intermediate forms transitional from Acephalina to Cephalina '.

It may not be out of place to review here in brief a few of the salient features of *Lecudina* which is the best known genus in the family Lecudinidæ Kamm as emended by Reichenow. Of the six genera which have so far been placed in the family, spore formation has been observed only in *Lecudina*. Regarding the others their inclusion in the family can only be provisional (until their sporocyst structure is known).

The early part of the life-history of *Lecudina* is intracellular as observed in *L. pellucida* (Léger, 1893; Ganapati, 1946), *L. elongata* (Brasil, 1909), *L. brasili* (Ganapati and Aiyar, 1937) and a new species of *Lecudina* (Ganapati, 1946). If normally present, and I am inclined to believe it is, this is an important character of the genus, inviting comparison with the Stenophoridæ Léger and Duboscq Cephaloidophoridæ Kamm and Monoductidæ Ray and Chakravarthi, all of which are septate families of gregarines.

The epimerite in all the known species of *Lecudina* is simple symmetrical and deformable, ranging from a simple small papilla in *L. elongata* to a long proboscis ending in a mucron in *L. longicephala* (Ganapati, 1946). In those forms where the epimerite is described as intracellular and caducous, confirmation is lacking and, until this is forthcoming, they may be left out of consideration. In general, it may be stated that the epimerite of *Lecudina* is in marked contrast with the same structure in the large majority of the Cephaline gregarines, where it is a permanent and, in many cases, a complex apparatus which is shed before association and encystment.

The differentiation of the protoplasm at the anterior end from that of the remainder of the body is another feature present in all the species which have been carefully studied; this seems to be characteristic of the genus and according to Kamm ' is an important one in assigning the family Lecudinidæ to an intermediate position between the monocystids and the polycystids'. This differentiation which may be overlooked owing to faulty technique in preservation or staining, cannot be missed in fresh preparations where it is very striking.

The association, cyst formation and spores are known only in four species of the genus and in general it may be stated that the sexual cycle closely resembles and conforms to the polycystid type. While the cyst formation occurs within the host, its further development may take place either within the host or outside in the sea water. In all the three forms studied by me cysts complete their development outside the host. The spores of all the known forms are ovoidal with a characteristic thickening at one pole and they are liberated by simple dehiscence of the cysts.

Summary

1. The detailed morphology and life-history of a dicystid gregarine, ***Lecudina pellucida*** (Köll.) Mingazzini, from the gut of ***Nereis chilkæensis*** Southern, are described.

2. The young stages of the parasite are intra-epithelial while the adult trophozoites lie free in the lumen of the gut attached to the intestinal wall by a sucker-like epimerite.

3. Cyst-formation takes place in the mid-gut and further development of the cysts takes place outside the host.

4. Gametogenesis is completed in about 48 hours. The gametes are anisogamous.

5. Spore-formation takes place in about 3 days and the spores are scattered by simple rupture of the cyst. The sporocysts are ovoidal with a thickening at one pole. There are eight sickle-shaped sporozoites and a spherical sporocyst residuum.

6. The systematic position of the genus *Lecudina* Mingazzini and of the family Lecudinidæ Kamm are discussed in the light of these observations.

Acknowledgments

My thanks are due to Professor R. Gopala Aiyar under whose direction the work was carried out and to the authorities of the University of Madras

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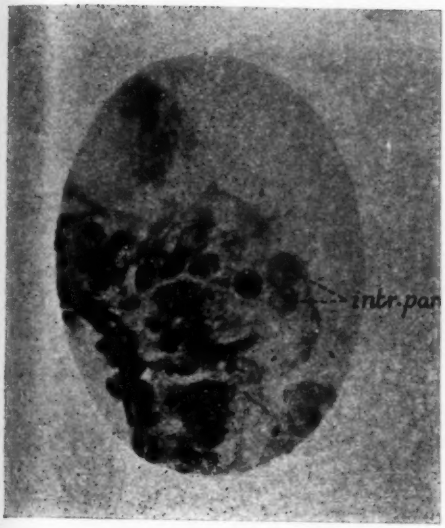

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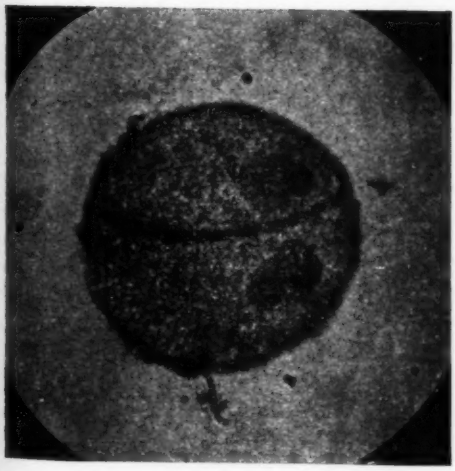

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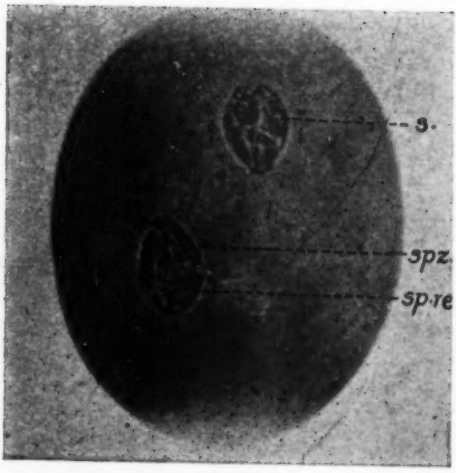

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Explanation of Plate

Photomicrograph 1. T. S. of gut epithelium showing intracellular stages of the parasite.

„ 2. An adult trophozoite attached to the gut.

„ 3. Section of a cyst soon after it is passed to the outside.

„ 4. Two sporocysts. Note the sporozoites, the sporocyst residuum and the thickening of the sporocyst wall at one pole.

Key to Lettering

intr. par. Intracellular parasite.
ep. Epimerite.
s. Sporocysts.
spz. Sporozoites.
Sp. res. Sporocyst residuum.

NOTES ON SOME GREGARINES FROM POLYCHÆTES OF THE MADRAS COAST

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Introduction

I propose to give in this paper a few preliminary observations on some Gregarines from Polychætes encountered while engaged in a more intensive study of other sporozoans from the hosts concerned. Considering that so little is known about Gregarines from polychætes, especially from India, I hope these preliminary observations will be of some interest.

The present paper records ten new species with new records of hosts for two known species.

Setna (1931) described two new Gregarines, *Bhatiella morphysæ* and *Ferraria cornucephali* from the gut of a polychæte *Marphysa sanguinea* Montague, taken at Port Blair in the Andamans. Ganapati and Aiyar (1937) recorded the genus *Lecudina* for the first time from India and described a new form *Lecudina brasili* from the gut of *Lumbriconereis* sp., from Madras. Bhatia and Setna (1938) later recorded five more genera comprising of six species of Gregarines from polychætes collected at Andamans, of which two are described as new to science. While this is the position regarding the Eugregarines, there is only a single form of Schizogregarine recorded by the above authors (Bhatia and Setna, 1938,) *Selenidium amphinomi*, from the cœlomic cavity of *Amphinoma rostrata* (Pallas), also taken at Port Blair in

the Andamans. Apart from these contributions, I am not aware of any other work on Gregarines from Indian polychætes.

The present paper describes forms belonging both to the Eugregarinaria Doflein and the Schizogregarinaria Leger. In the case of the Eugregarines the adult trophozoites present a few definite morphological features which are of value in determining their systematic position. The Schizogregarinaria, however, still remain a heterogenous assemblage of animals and most of the genera included in this group require further investigation. The best known genus is ***Selenidium*** Giard. Ray (1930) by redescribing several imperfectly known species of ***Selenidium*** and many new ones from polychætes, has shown that the trophozoites possess certain definite morphological features which are of taxonomic value. The most important of these is the constant presence of a few 'chromatic bodies' at the anterior end of the parasite in all the species studied by him. He has further shown that Schizogony, which is supposed to be an important feature in the definition of the Schizogregarinaria need not in all cases be present and, in his opinion, the genus itself requires drastic revision and may have to be dismembered in the light of future work.

Material and Methods

Of the nine species of worms examined, all excepting one occur in Madras and its suburbs. Observations on the parasite from *Platynereis abnormis* (Horst) were made during two short visits to the Krusadai Islands, in the Gulf of Mannar, during the years 1939 and 1940 and on preserved material collected.

The techniques employed are essentially the same as those described in a previous paper (Ganapati and Aiyar, 1937). In describing the forms emphasis has always been laid on the examination of living material which brings forth some important features better than the preserved material and sections.

Family Lecudinidæ Kamm
emend Reichenow, 1929

Lecudina krusadiensis n.sp. (Figs. 1–3)

Host.—*Platynereis abnormis* (Horst).

Habitat.—Intestine.

Locality.—Krusadai Island, Gulf of Mannar, *India.*

The host worms are fairly abundant amidst sea-weeds at Sandy Point. If the weeds are vigorously shaken in a basin of water the worms emerge and

actively swim about. Almost all the worms examined harboured the parasite, in the mid and hind regions of the gut, and in heavily infected worms, a few may be found in the anterior region also.

The trophozoites are elongate, cylindrical and broadly tapering at the extremities. Attachment to the gut wall is effected by a button-like epimerite closely applied to the gut epithelium. Behind the epimerite there is a clear hyaline zone of cytoplasm distinct from the thickly granulated cytoplasm of the main body. Both in fresh and permanent preparations the epimerite is transparent, but in the region behind, a few deeply staining longitudinal striations are seen. The epimerite is retractile into the anterior end and attachment is effected by a sucker-like action. The pellicle is fairly thick and in sections the parasite is rounded.

The nucleus is large and spherical containing a single karyosome. The chromatin forms a thick network outside the karyosome.

The living parasite executes active gliding movements. The animal is capable of bending on itself and suddenly straightening with a jerk. In these movements the anterior region shows more flexibility than the posterior. The animal effects slight changes in bodily shape by contraction and elongation in an antero-posterior axis.

A few advanced intra-epithelial stages of the parasite have been seen in sections and there is no doubt that the early young stages are intracellular (Fig. 1). The smallest such stage measured 22 by 10 microns. The body is pear-shaped and the epimerite already differentiated, in the form of a hemispherical button. Further growth appears to be mainly in length and, ultimately, the host cell is ruptured and the adult animal hangs freely into the gut attached by the epimerite.

Cysts and spores are not known.

Measurements.—Length of trophozoite 100 to 150 microns; width 20 to 25 microns; diameter of nucleus 10 microns.

Remarks.—This species invites comparison with ***Lecudina elongata*** (Mingazzini) in the shape of the trophozoite and nature of the epimerite, which Mingazzini describes as a 'picolo botton sferico'. According to Reichenow (1932) an epimerite is absent in ***L. elongata*** and the animal is described as attaching itself to the gut epithelium by its differentiated anterior end functioning like a sucker. Both the above authors illustrate the differentiation of the body protoplasm at the anterior end in ***L. elongata.*** The trophozoites in ***L. krusadiensis,*** however, do not attain such large dimensions as ***L. elongata*** (500 by 40 microns) and the structure of the nucleus is also

different containing only a single karyosome instead of several as in the latter. These two features and the difference of the host are taken into consideration in describing the Gregarine from *Platynereis abnormis* as a new species.

Lecudina indica n.sp.

(Figs. 7–13)

Host.—*Lycastis indica* Southern.

Habitat.—Intestine.

Locality.—*Madras*, Madras.

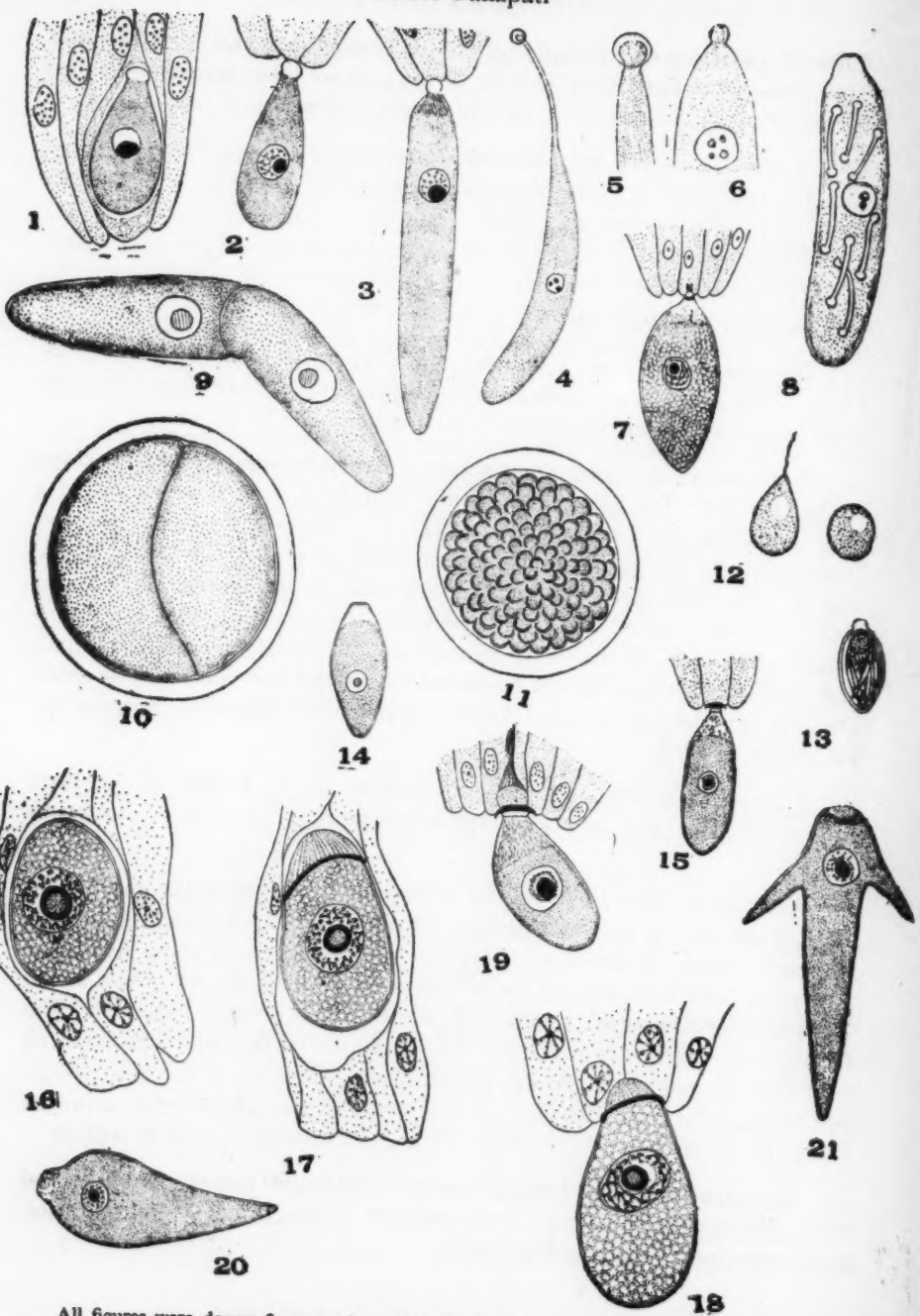
The host worm occurs burrowing in mud along the banks of Buckingham Canal. The parasites are fairly common, especially in the young and medium-sized worms, confining themselves to the hind gut.

Trophozoites are long and cylindrical, broadly tapering at the extremities (Fig. 7). The epimerite is in the form of a short cone ending in a hyaline papilla, and attachment is effected by a sucker-like action. The free trophozoites in the gut showed different stages in the retraction of the epimerite. The body protoplasm is differentiated at the anterior end into a finely granulated zone which, in living parasites, appears hyaline and transparent. The rest of the body protoplasm is coarsely alveolate. Hyperparasites are often seen in the body and when these are numerous the body shape is considerably altered. These hyperparasites appear to be *Metchnikowella* sp. (Fig. 8).

The nucleus is large, spherical and situated in the middle of the body and it contains one or two large karyosomes and a cloud of chromatin outside.

Association takes place by the anterior ends, but later becomes lateral (Figs. 9 and 10). Cysts are spherical and are passed to the outside along with the fæcal pellets of the worm. Further development and spore formation take place outside the host in about 3 days time. Gametes are of two kinds. The male is pear-shaped, motile and provided with a single flagellum. The female is non-motile and rounded. Sporocysts are ovoidal with a pronounced thickening at one pole. Spores are octozoic containing a spherical sporocyst residuum at one pole (Fig. 13). Sporozoites are sickle-shaped, arranged along the longer axis of the spore. The cysts dehisce by simple rupture.

Measurements.—Trophozoites measure 100 to 190 microns in length and 30 to 50 microns in width. Cysts measure 40 to 70 microns in diameter. Sporocysts measure 12 by 8 microns.



All figures were drawn from sections with the aid of a camera lucida at stage level, with Zeiss apochromatic oil immersion objective and compensating oculars. Unless otherwise stated,

the material was fixed in alcoholic Bouin–Duboscq and the sections stained in Heidenhain's iron-hæmatoxylin. The magnifications given are those at which the drawings were made but figures 1–35 have been reduced to half.

Lecudina krusadiensis N.Sp.

Fig. 1. An advanced intra-epithelial parasite × 1800.
Fig. 2. A young trophozoite attached to the gut epithelium × 840.
Fig. 3. A full-grown trophozite × 840.

Lecudina longicephala N.Sp.

Fig. 4. A trophozoite with the epimerite in a fully extended condition. Note the cup-shaped epimerite at the anterior end. × 280.
Fig. 5. A magnified anterior end of the epimerite (fresh) × 800.
Fig. 6. Anterior half of a trophozoite showing the retracted condition of the epimerite (fresh) × 800.

Lecudina indica N.Sp.

Fig. 7. A young trophozoite attached to the gut epithelium × 1350.
Fig. 8. An adult trophozoite which is hyperparasitised. Note the rod-shaped bodies in the cytoplasm and the differentiation of the protoplasm in the anterior zone from the rest of the body (fresh) × 400.
Fig. 9. Associating sporonts (fresh) × 400.
Fig. 10. A cyst (fresh). Note the thick ectocyst × 800.
Fig. 11. A fresh gametocyst × 800.
Fig. 12. The male and female gamets (fresh) × 1800.
Fig. 13. A sporocyst (fresh). Note the sporozoites and sporocyst residuum. × 1800.

Polyrhabdina minuta N.Sp.

Fig. 14. A free trophozoite from the lumen of the gut (fresh). Note the clear zone of cytoplasam at the anterior end × 400.
Fig. 15. A trophozoite attached to the epithelium of the host. Note the epimerite. × 400.

Sycia polydoræ N.Sp.

Fig. 16. An early intra epithelial parasite × 1800.
Fig. 17. A later stage showing the epimerite × 1800.
Fig. 18. A young trophozoite attached to the gut wall × 1800.

Sycia legeri N.Sp.

Fig. 19. A trophozoite attached to the gut epithelium × 1800.

Ancora sagitata (Leuck.)

Fig. 20. A ripe sporont × 400.
Fig. 21. A trophozoite. Note the depression and differentiation of the protoplasm at the anterior end. × 800.

Remarks.—L. indica resembles *L. pellucida* in the nature of the epimerite. The sporocysts are larger than in *L. pellucida* and the polar thickening more pronounced. The structure of the nucleus is also different.

Lecudina longicephala n.sp.

(Figs. 4 to 6)

Host.—Pisionidens indica Aiyar and Alikunhi.

Habitat.—Intestine.

Locality.—Madras, Madras.

The host worms occur in the intertidal zone of the Madras foreshore. Infection is rare and when present only a few parasites were observed in the host.

The trophozoite is elongate and cylindrical with the posterior end rounded. The anterior end is drawn out into a long slender proboscis ending in a cup-shaped organ of attachment or epimerite (Photomicrograph 1). The body protoplasm is thickly granular while the protoplasm in the proboscis is hyaline in living parasites. The epimerite is also transparent. The proboscis is completely retractile into the anterior end and in this condition it is visible as a knob at the anterior end.

The nucleus is large and spherical with 3 to 6 rounded karyosomes.

The free trophozoites show active gliding movements. Changes in body shape are also effected by contraction and elongation in an antero-posterior axis. There is a layer of well-developed circular myonemes below the pellicle, and these are probably related with the active movements of the animal.

Cysts and spores not known.

Measurements.—Length of trophozoite including epimerite 400 to 630 microns; epimerite alone 216 microns; width of trophozoite 50 microns; diameter of nucleus 26 microns.

Remarks.—The epimerite in *L. longicephala* resembles to some extent the same organ in *L. aphroditæ* (Lank.) and *L. lysidicæ* Bhatia and Setna. In both these forms a cup-shaped mucron has not been described and the shape of the trophozoite is also entirely different. In *L. lysidicæ* the epimerite is described as caducous, the sporonts being without it. My observations on the present form show that the only part of the epimerite which is caducous is the mucron while the rest of the proboscis is completely retracted and probably absorbed by the sporonts. A comparison with *Ferraria comucephali* Setna, is also difficult as in this form the epimerite is described as 'a wide-mouthed funnel-like structure on a long slender tubular stalk'. I have nothing to show that the epimerite in the present form is tubular. The mucron appears to be a circular cup-shaped expansion of the pellicle at the anterior end of the stalk, functioning as a sucker for attachment. Until something is known about the sexual stages and the sporocyst structure it appears to me reasonable to include it in the genus *Lecudina* as a new species.

A tabulated statement showing the diagnostic features of all known species of *Lecudina* is given at the end of this paper. All are from the intestine of the hosts concerned.

Polyrhabdina minuta n.sp.

(Figs. 14 and 15)

Host.—*Prionospio cirrifera* Wiren.

Habitat.—Intestine.

Locality.—*Madras,* Madras.

The host worms are found burrowing in mud at the mouth of river Cooum. The percentage of infection is very small and in no case was it heavy. The parasite lives in association with another schizogregarine, in the hind gut, which is described elsewhere in this paper.

The trophozoites are elliptical with the anterior end drawn out into a short neck and posterior end tapering to a fine point. The epimerite is in the form of a short cylinder which spreads out into eight prongs or claws firmly embedded in the gut epithelium of the host. The free parasites are without the epimerite, they being left behind when the animal breaks away from their attachment. The epimerite is colourless in fresh preparations but is stained deep with hæmatoxylin and pink with Mann's stain. Behind the epimerite there is a clear zone of cytoplasm which stands in distinct contrast with the thickly granulated general body protoplasm. There is no septum between these two regions as in polycystid Gregarines. The pellicle surrounding the body is thick and there are well marked longitudinal epicytal striations running along the length of the body.

The nucleus is spherical with a single large karyosome.

The free sporonts in the gut execute active gliding movements, but there is no change in body shape owing to the thick nature of the pellicle. The body of the trophozoite often harbours hyperparasites probably belonging to species of *Metchnikowella.* When these are present the parasite assumes various shapes and deformities.

Measurements.—Length of trophozoite 30 to 60 microns; width 20 microns.

Remarks.—The present form resembles *Polyrhabdina spionis* (Koll.) as figured by Caullery and Mesnil (1914) from *Scolelepis fuliginosa.* The trophozoites are, however, much smaller and the epimerite prongs simple and not bifurcate as in the above form. The presence of a septum in *Polyrhabdina* as described by Kamm (1922) is considered an error by Reichenow (1929) the septate appearance, according to this author being due to the finely granulated nature of the protoplasm at the anterior end, compared with the posterior region. He has therefore amalgamated the family Polyrhabdinidæ

with Lecudinidæ. Mackinnon and Ray (1931) have not seen a septum in the form described by them and I have already stated there is no septum in the present form also. An intracellular phase in the development has not been seen by me and this substantiates the view expressed by Mackinnon and Ray that the whole development may be extracellular. The smallest forms observed by me were minute bottle-shaped animals clinging to the epithelium by well-formed epimerites.

Sycia legeri n.sp.

(Fig. 19)

Host.—Cirratulus filiformis Keferstein.

Habitat.—Intestine.

Locality.—Madras, Madras.

The host worms occur in large numbers among debris over shells of oysters and in crevices of stones in the Madras Harbour. The parasite lives in association with three other sporozoans, a Coccidian, two species of a schizogregarine and an astomatous ciliate.

The trophozoites are ovoidal or fiask-shaped. The epimerite is a hemispherical knob surrounded at the base by a ring-like collar. The collar which has a sharp-edged shelf all round is firmly embedded in the gut epithelium. The epimerite is completely intracellular. In living parasites the knob is transparent and hyaline while the body protoplasm is differentiated into two distinct regions. The anterior region behind the epimerite is clear and free of granules while the protoplasm behind in the main body is granulated. There is, however, no septum between these two regions.

The nucleus is spherical with a single large karyosome.

The free sporonts are without the epimerites.

Measurements.—Length of trophozoite 20 to 40 microns; width 10 to 15 microns.

Remarks.—Reichenow (1932, 1935) in giving an emended definition of the genus Ulivina, considers that *Sycia inopinata* Leger is identical with *Ulivina elliptica* Mingazzini. However, the important generic character, namely, the knob-shaped epimerite surrounded at the base by a ring-like collar, as described by Leger is not shown by all the three species of *Ulivina* described (Mingazzini, 1891; Kamm, 1922; Bhatia, 1938). Bhatia therefore considers that the two genera are distinct and I am led to agree with the above view.

Leger's figure of *Sycia inopinata* from the intestine of *Audouinia* (Cirratulus) *Lamarkii* shows a distinct septum and Kamm evidently following Leger's description, places the form among the septate Gregarines. I am inclined to consider that this may be an error in observation owing to the differentiation of the protoplasm in the two regions, as shown in the present form, and that *Sycia* is as much a dicystid as *Polyrhabdina*. The real position of *Sycia* should therefore be in the family Lecudinidæ as amended by Reichenow (1929).

Sycia polydoræ n.sp.

(Figs. 16–18)

Host.—*Polydora ciliata* Johnston.

Habitat.—Intestine.

Locality.—*Madras*, Madras.

The host worms are found boring in shells of oysters in the San-Thome Bridge area, Adyar. The parasite lives in association with a Coccidian described elsewhere (Ganapati, 1939) and a Schizogregarine described in this paper.

The trophozoites are flask-shaped with the anterior end slightly drawn out into a short neck. The epimerite is in the form of a knob surrounded at the base by a deeply staining collar. The whole epimerite is completely embedded in the epithelium of the host's gut. The body protoplasm is differentiated into two regions, an anterior zone where the cytoplasm is clear and a region behind which is thickly granulated and alveolar.

The nucleus is large and spherical containing a single karyosome. The karyosome shows an outer deeply staining rind and an inner lighter medulla. The chromatin forms a thick network.

The young stages are intracellular within the gut epithelium. The earliest stage observed measured 25 by 18 microns and was ovoidal in shape. An epimerite was absent at this stage, but in a later stage while the parasite was still intracellular a well-developed epimerite was present (Figs. 16 and 17). The epimerites are lost when the animal becomes free and in sections these discarded epimerites are seen clinging to the gut epithelium of the host.

Measurements.—Length of trophozoite 35 to 80 microns; width 18 to 20 microns; diameter of nucleus 10 microns.

Remarks.—Leger (1893) described a dicystid Gregarine *Doliocystis polydoræ* from the gut of *Polydora agassizii*. The epimerite is described as ' un tronc de cone á petite base inférieure ' continued directly from the anterior

extremity of the second segment which is elongated in the form of a neck. The epimerite is said to be intracellular and caducous. Caullery and Mesnil (1914) when describing a species of *Polyrhabdina* from *Spio martinensis* state that it has an epimerite of fundamentally the same type as that of *Polyrhabdina spionis* and add that 'il en est sans doute de meme de l'epimerite du *Doliocystis polydoræ* décrit par Léger; nous avons pu le verifier pour une Gregarine de *Polydora ciliata*'. In the list of species of *Polyrhabdina* they insert '*P. polydoræ* (Leger) de *Polydora ciliata*'. *Polydora agassizii* is a variety of *Polydora ciliata*. Kamm (1922) records this form as a *Doliocystis*, and names it *Lecudina polydoræ* (Leger). Mackinnon and Ray (1931) while describing other Gregarines from polychætes came across a form in the gut of *Polydora flava* which they consider may be the species observed by Caullery and Mesnil and on their authority describe it as *Polyrhabdina polydoræ* Caul. and Mesn. (?). The epimerite in this form has a knob-like portion embedded in the host's tissue and a collar surrounding its base. In front of the collar is a circlet of prongs which firmly attach the Gregarine to the host cell. The resemblance between this type of epimerite and the epimerite of *Sycia* described by Leger (1892) and of the present form is striking. The circlet of prongs above the collar is not present in *Sycia* as in *Polyrhabdina*. It is unfortunate that Leger has not given any figure of the parasite from *Polydora agassizii* and with the meagre description available it is not possible to say whether the parasite described by him is identical with the present form.

Ancora sagittata (Leuck.)

(Figs. 20 and 21)

Host.—*Capitella* sp.

Habitat.—Intestine.

Locality.—*Madras*, Madras.

The host worms occur in small numbers along the banks of the river Cooum near its mouth.

The trophozoites have the characteristic shape of an anchor with the antero-lateral horns (Photomicrographs 3 and 4). The anterior end is dome-shaped with a cup-shaped concavity at the point of attachment of the parasite to the host's gut. The body tapers to a point posteriorly. The cytoplasm is finely granular, and the body is surrounded by a thick pellicle.

The nucleus is spherical and is placed in the middle of the body between the antero-lateral prolongations where the body is widest. There is a single rounded karyosome and a few grains of chromatin in the nuclear sap. The

karyosome shows an outer deeply staining rind or cortex and an inner lighter medulla.

The attachment to the host epithelium is effected by a sucker-like action of the anterior end. The cytoplasm shows a clear zone at this end in the living parasite. The cup-shaped appearance is produced by the retraction at the anterior end which forms a vesicle and functions as the epimerite.

The body of the trophozoite often harbours what appear to be stages in the development of a hyperparasite, probably a species of *Metchnikowella*. The free sporonts are without the antero-lateral horns, and these are presumably absorbed into the general body protoplasm before association and cyst formation. The free trophozoites show active gliding movements. The antero-lateral horns are used as paddles in these movements.

Measurements.—Length of trophozoite 100 to 150 microns; width 25 to 30 microns.

Remarks.—The present parasite resembles *Ancora sagittata* (Leuck.) which Cecconi (1905) describes from the gut of *Capitella capitata*. Cecconi describes and figures the earlier stages of the parasite as intra-epithelial. I have not come across these stages, and my observations show that the epimerite is not intracellular, as stated by Cecconi, but lie completely outside the host's tissue functioning as an adhesive sucker-like vesicle. Reichenow (1929) has recently placed the genus *Ancora* Labbe in the family Lecudinidæ Kamm as emended by him.

SUB-ORDER SCHIZOGREGARINARIA Leger, 1900

Genus *Selenidium* Giard, 1884

emend Brasil, 1907 and Ray, 1930

Selenidium pendula Giard

(Fig. 31)

Host.—*Nerine cirratulus* Claparede.

Habitat.—Intestine.

Locality.—*Madras*, Madras.

The host worms are found burrowing in sand in the intertidal zone of the Madras foreshore. They are present only in small numbers and out of a dozen worms examined only one was infected and contained three parasites.

The trophozoites attain large dimensions up to a size of 300 to 400 microns in length and 30 to 35 microns in width. Anteriorly the body is bluntly rounded while posteriorly it tapers to a fine point. There are both

longitudinal and circular striations on the body. Just behind the anterior end there is a small zone which is free of striations. In living parasites this area appears clear and hyaline. The body protoplasm is granular.

The nucleus is ovoidal and elongated parallel to the long axis of the body. The karyosome is rounded and closely applied to the nuclear membrane at one end, while the chromatin forms a spindle-shaped mass in the centre of the nucleus. There is a clear space between the nuclear membrane and the chromatic mass.

The living parasites show the characteristic serpentine movements of the genus.

Remarks.—Giard founded the genus *Selenidium* in 1884, and described the type species *S. pendula* from the body-cavity of *Nerine* sp. Caullery and Mesnil (1899) rediscovered the form described by Giard in the digestive tract of *Nerine cirratulus* and corrected Giard's mistake regarding the habitat of the parasite. Brasil (1909) added a few further observations on the trophozoite of the same form and found that the association took place by the posterior ends. An intracellular schizogony and sporocyst formation have not so far been observed in this type species of the genus and this point must be cleared before dismembering the genus itself as suggested by Ray (1930, 1933).

Selenidium polydoræ n.sp.

(Figs. 22 and 23)

Host.—Polydora ciliata Johnston.

Habitat.—Intestine.

Locality.—Madras, Madras.

The host worms occur boring in shells of oysters in the brackish-water pools of Adyar. A coccidian and a dicystid Gregarine have already been described from the same host.

The trophozoites are long and slender measuring 100 to 150 microns in length and 8 to 12 microns in width. The body shows both longitudinal and circular striations. The living parasites execute active wriggling, twisting and serpentine movements. Attachment to the host epithelium is effected by a small nipple-like structure at the anterior end where the protoplasm is clear.

The nucleus is ovoidal and its longer axis lies parallel to the longer axis of the body. The karyosome is attached to the nuclear membrane at one end and the chromatin forms a finely granulated elliptical zone in the middle of the nucleus.

No intracellular schizogony has been observed although the serial sections of over 200 worms were examined in connection with the study of the Coccidian infecting the gut of the same host (Ganapati, 1939).

I have seen associating sporonts in which the nucleus multiplies precociously. Association is end to end to start with and the sporonts shorten until the line of apposition gradually becomes lateral (Photomicrograph 5). The completed cysts are passed to the outside and further development appears to take place in sea-water. I have not succeeded in tracing further development of cysts and spore formation.

Remarks.—Caullery and Mesnil (1899) observed two forms of *Selenidium*, one in the gut of *Polydora flava* Clpde. and the other in the gut of *Polydora cæca* Oerst. Fowell (1936) has recently redescribed the form in *Polydora flava* as *Selenidium axiferens* Fowell, owing to the presence of a characteristic 'axial organ' inside the body. Caullery and Mesnil had seen a similar structure in both the forms they described. I have not seen any such structure in the present form and as such it cannot be the species observed by the above authors. I am therefore describing the form from *Polydora ciliata* as a new species of the genus.

Slenedium cirratuli n.sp.

(Figs. 27 to 29)

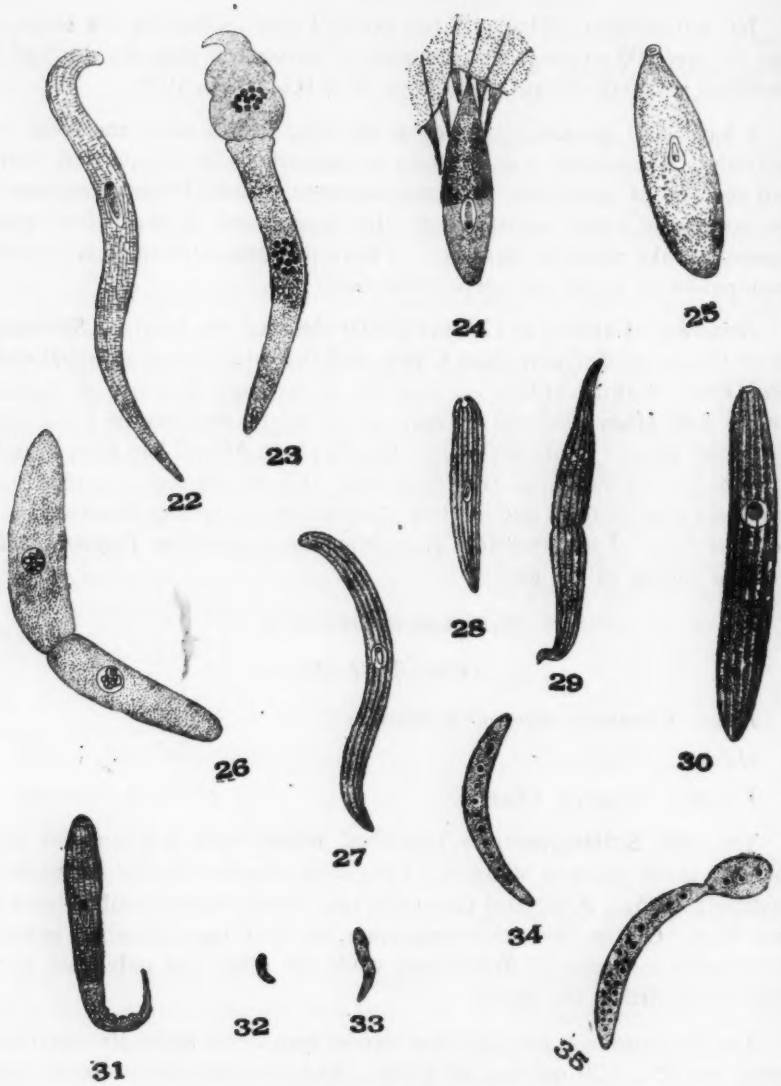
Host.—*Cirratulus filiformis* Keferstein.

Habitat.—Intestine.

Locality.—*Madras*, Madras.

The two Schizogregarines described below were encountered while making a more intensive study of a Coccidian infecting the gut of the worm (Ganapati, 1945). A dicystid Gregarine has already been described from the same host. Of the two schizogregarines the first one described below is undoubtedly a species of *Selenidium*, while the other has only been provisionally included in the genus.

The trophozoite is elongate and slender measuring 80 to 100 microns in length and 15 to 20 microns in width. The anterior end is blunt while posteriorly the body is gradually tapering. There are 10 to 12 longitudinal epicystal striations running along the whole length of the body, excepting for a clear zone at the anterior end, which is knob-like and retractile. The attachment to the gut epithelium is effected by a sucker-like action of this end.



Selenidium polydoræ N.Sp.

Fig. 22. A trophozoite × 900.
Fig. 23. Associating sporonts. Note the multi-nucleate condition. Smear. × 1200.

Selenidium sp.

Fig. 24. Section of a young trophozoite attached to the gut epithelium × 1200.
Fig. 25. A free trophozoite. Note the epimerite. × 400.
Fig. 26. Associating sporonts (Smear) × 900.

Selenidium cirratuli N.Sp.

Fig. 27. A fresh trophozoite. Note the differentiation of the protoplasm at the anterior end. × 600.

Fig. 28 A trophozoite showing the retracted condition of the epimerite (fresh) × 600.

Fig. 29. Associating sporonts (fresh) × 600.

Selenidium spionis N.Sp.

Fig. 30. A trophozoite. Note the concavity at the anterior end (Smear) × 900.

Selenidium pendula Giard.

Fig. 31. A trophozoite (Smear) × 200.

Siedleckia nematoides Caull. & Mesn.

Fig. 32. A single-nucleated parasite (Smear) × 900.

Fig. 33. A parasite with three nuclei (Smear) × 900.

Fig. 34. A female (?) with nuclei arranged in linear series (Smear) × 900.

Fig. 35. A male (?) showing a multi-nucleate condition at one end (Smear) × 900.

The nucleus is ovoidal and slightly elongate along the long axis of the body. There is a single karyosome and a mass of chromatin granules in the centre.

Association takes place by the anterior ends, the epimerites probably serving to hold together the sporonts till association is completed. The nucleus becomes spherical in the sporonts. A few round cysts measuring 70 microns in diameter have been observed which might belong to either of the two forms. The cysts are passed to the outside along with the fæcal matter of the worm and their further development, which has not been followed, presumably take place in the sea-water.

Remarks.—Caullery and Mesnil (1899) observed two species of *Selenidium* in *Cirratulus cirratus* Muel. and called one 'en virgule' or comma shaped, and the other 'point et virgule' or shaped like a semicolon. A totally different form *Selenidium plicatum* was described by Ray (1930) from the same host. The form described above may be Caullery and Mesnil's comma-shaped form.

Selenidium spinosis n.sp.

(Fig. 30)

Host.—Prionospio cirrifera Wiren.

Habitat.—Hind-gut.

Locality.—Madras, Madras.

The host worms are gregarious occurring in large numbers along the banks of the river Coum. Only very rarely the worm harbours the schizogregarine.

The trophozoites are vermiform measuring 60 to 80 microns in length and 10 to 12 microns in width. The anterior end is bluntly rounded while

posteriorly the body is abruptly tapering. There is a shallow sucker-like depression at the anterior end which functions as the epimerite. The whole length of the body is traversed by about 10 broad epicytal striations. The nucleus is spherical with the main nuclear mass consisting of the karyosome and the ball-shaped chromatin lying in the centre. Associating forms and cysts are not known.

Selenidium sp.

(Figs. 24 to 26)

Host.—Cirratulus filiformis Keferstein.

Habitat.—Mid and hind-gut.

Locality.—Madras, Madras.

The second kind of schizogregarine in the worm can be distinguished from the first already described by the broader and shorter nature of the trophozoite, and the absence of the characteristic epicytal striations on the body. The anterior end is slightly drawn out into a short neck which terminates in a cup-shaped sucker or epimerite. The body protoplasm is finely granulated. The nucleus presents the typical structure seen in *Selenidium.* Associating sporonts and cysts have been observed, the latter being passed along with the fæcal matter of the worm. Until something more is known about this it is provisionally referred as a species of *Selenidium.*

Siedleckia nematoides Caullery and Mesnil

(Figs. 32 to 35)

Hosts.—Scoloplos sp.

Habitat.—Hind-gut.

Locality.—Madras, Madras.

The host worm occurs in the brackish-water pools of Adyar.

The trophozoites are nematode-like vermiform bodies and in living parasites the body is hyaline. The parasites show active twisting and serpentine movements like species of *Selenidium.* The trophozoite measures 40 to 70 microns in length and 7 to 11 microns in width. Usually the body contains several nuclei which is characteristic of the genus. Single nucleated forms are rare and requires careful search. Two types of multi-nucleate forms are seen. In one the nuclei are all arranged in a linear series while in the other one end of the parasite contains a large number of nuclei without any arrangement. The nuclei are dense, rich in chromatin and stain deep with hæmatoxylin. There is a clear space between the nucleus and the body protoplasm.

Remarks.—Caullery and Mesnil who were the first to observe these parasites in the intestine of *Scoloplos armiger* considered them as aberrant sporozoans. Labbe (1899) considered them intermediate between Gregarines and Mesozoans. Leger (1907), Leger and Duboscq (1910) and Minchin (1912) have described them as schizogregarines and placed them near the genus *Schizocystis*. Recent researches of Chatton and his collaborators (1929–1936) on three more sepcies of the genus have shown that in reality the two kinds of parasites represent the sexual forms and that the budding observed by Caullery and Mesnil, represent in reality stages in the formation of the gametes, which were mistaken by the above authors as a process of asexual multiplication or schizogony.

The parasite with nuclei arranged in a linear series is the female which buds off a number of uni-nucleate bodies or macrogametes, while the form with a multinucleate end buds off multinucleate bodies, each of which produces a few male gametes. The authors describe a typical fertilisation process between the gametes and formation of cysts. This type of gametogenesis and fertilisation is unique among the Gregarines and invites comparison with the Coccidians.

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Summary

1. Four genera belonging to seven species of Eugregarines and two genera comprising of five species of Schizogregarines are described from some common polychætes found at Madras. The hosts examined include nine genera of as many species.

2. Of the seven Eugregarines described six are new to science, and all of them come under the family Lecudinidæ Kamm emend Reichenow, 1929. The three genera, *Polyrhabdina*, *Sycia* and *Ancora* are recorded for the first time from India. The retention of the genus *Sycia* Leger as different from *Ulivina* Mingazzini, and its inclusion in the family Lecudinidæ have been suggested.

3. The Schizogregarines described come under the genera *Selenidium* Giard and *Siedleckia* Caull. and Mesn. Four new species of *Selenidium* are described while the fifth one has been provisionally assigned to this genus. The genus *Siedleckia* is a new record from India.

Acknowledgments

I wish to thank Professor R. Gopala Aiyar for his many useful suggestions and criticisms and the Syndicate of the Madras University for providing

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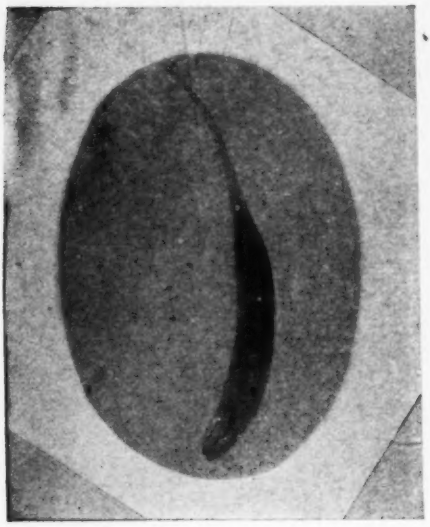

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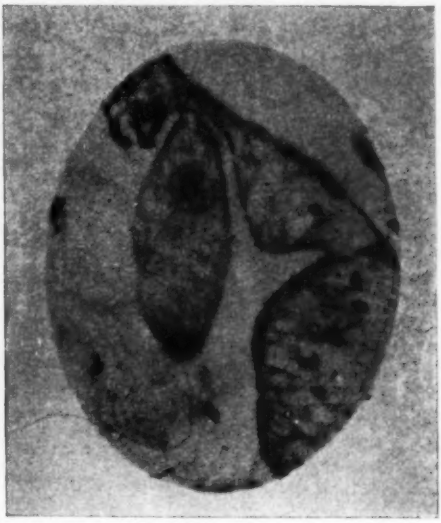

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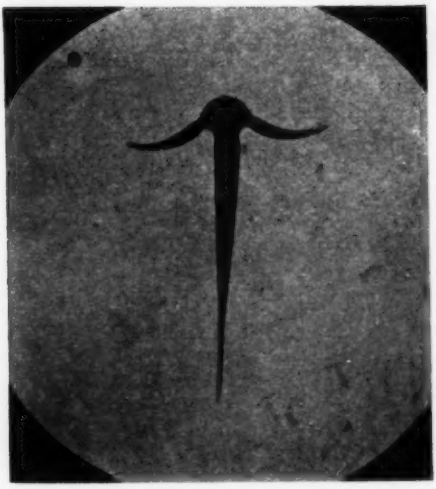

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Photo-micrograph 1. *Lecudina longicephala* (Smear)
„ 2. *Sycia polydorae* (Section)
„ 3. *Ancora sagittata*. Trophozoite (Smear)
„ 4. *Selenidium polydorae*. Associating sporonts

DEVELOPMENTAL STUDIES

I. Origin and Development of Axillary Buds with Special Reference to Two Dicotyledons

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Received October 29, 1945

Introduction

Buds occur in the axils of leaves of almost all plants which expand by lateral branching. It is the prevailing condition in Ferns, Gymnosperms and Angiosperms. Nägeli distinguished two kinds of buds, namely, *acrogenous* and *phyllogenous* (axillary), and ascribed the latter mode of origin to Equisetaceæ and Spermophyta (Goebel, ii, p. 432, 1905).

Recently Wardlaw (1943, '44) studied the origin and development of buds in some members of the Leptosporangiate Ferns. He showed that even the extra-axillary buds in many Ferns are essentially axillary in origin. This he established only by developmental studies. He further showed that in the majority of cases studied by him the initiation of bud takes place in the *detached meristem*[1] on the free surface of the shoot at some distance behind the growing apex.

So far as the present writers are aware, not much work has been done on the initiation and positional relationship of the axillary buds in the Gymnosperms. Besides, the majority of leaves on a conifer, such as Spruce, do not bear axillary buds.

In the Monocotyledons the developmental studies of the shoot apex is a recent one. Skutch (1927), Rösler (1928), Kliem (1937), Sharman (1942, 1945) and Hsü (1944) studied the initiation of axillary buds in Banana, *Triticum*, *Avena*, Maize, *Agropyron* and Bamboo shoot apices respectively. Skutch states that the lateral buds do not occur in the axils of leaves as reported previously by Schumann but are " situated opposite them ", that is, opposite the leaves which are concerned with the origin of the axillary buds. He says that these buds regularly appear in the angles of the V formed by the two margins of the sheath as they converge to the point of insertion

[1] By *detached meristem* Wardlaw (1943 *a*) understood " the detached portion of the apical meristem " (p. 180) at some distance below the extreme apex.

(pp. 341–42). Sharman clarified the apparent obscurity in the statement of Skutch. He showed, perhaps for the first time, that the axillary bud is really " to be associated with the leaf above and its continued marginal growth and not with the leaf in whose axil it appears " (p. 254). This appears to be exactly what Skutch meant by " situated opposite them ". In explaining the origin of the bud Sharman says that " the final development of the bud represents the last phase of the growth which started on one side of the apex at the point where the leaf was initiated and passed down the leaf internode unit, and then reaches its conclusion in the formation of a bud on the other side of the stem ". Thus the observations of both Skutch and Sharman show that the axillant leaf in Banana and Maize has nothing to do with the origin of the bud in its axil.

Rösler (1928) and Kliem (1937) noticed bud initiation in the surface layer of the *corpus* in *Triticum* and *Avena* respectively. Hsü reports that in *Sinocalamus Beecheyana* the bud is initiated in the outermost layer of the peripheral zone of the *corpus* of the main shoot (pp. 408–09). This has been supported by Sharman (1945) in a later contribution though he gave a different name to this layer, namely, *subhypodermal layer*.

In Dicotyledons Koch (1893) was perhaps the first to study in detail the origin and development of axillary buds in *Syringa*. He reported that the initiation of an axillary bud is first recognised by vacuolating cells of the leaf primordia becoming more meristematic again and resuming active division. The activity then spreads into the axial tissue. Goebel in his *Organography* (1905, ii, pp. 432–33), writes, however, that the axillary shoots proceeds from groups of cells " of the axis of the shoot immediately above the insertion of the leaf, and these groups derived from the embryonal tissue of the vegetative points, have retained their embryonal character (*cf. detached meristem* of Wardlaw), but only at a later period are stimulated to a new formation into which also lower and already differentiated cells can be brought ". Louis (1935) figures the axillary buds of *Ranunculus repens* as confluent with the subtending leaf, though he states that the meristem is initially meristematic and does not redifferentiate from vacuolating cells; but in the absence of photographs or description of other stages it is not possible to see whether this is a disagreement of facts or interpretation. *Foliar origin* of axillary buds has been noticed in Buttercup grown wild in the University grounds at Leeds.

The origin of the *trace* in the axillary buds and its connection with the stele in the axis or the leaf-trace has also to be determined. Esau (1943 raises the question as to whether vascular connections between the bud and

the main axis or the axillary leaf, as the case may be, is basipetal or acropetal. She keeps the question open, for she says that if the concept of basipetal procambial differentiation of leaf-trace is applied to the problem of vascular development in axillary shoots, such shoots may be regarded as forming their connections with the main axis by a downward differentiation of their leaf-traces (see also Wardlaw, 1944). Wardlaw's (1944) observations on bud development in Ferns afford definite indications of a *basipetal* initial differentiation of the bud-trace. As Koch's original paper is not available to us it is not possible to say if he studied and reported on this problem. Skutch, Sharman and Hsü, so far as we could make out, did not study the vascular connections of the axillary buds.

Thus work so far done on the study of the origin and development of axillary buds and their vascular connections with the main axis in the major groups of plants appears to be quite meagre. In view of the importance of the study of this problem the writers have undertaken developmental studies of shoot apices of the dicotyledons first, and the present communication embodies the results of such studies of the shoot apices of *Heracleum* and *Leonurus*.

Origin and Development of Axillary Buds in *Heracleum sphondylium* L. and *Leonurus sibiricus* L.

Heracleum sphondylium L. is a herb with a perennial underground axis. Buds occur in the axils of nearly all older leaves, but of these only a few develop into active shoots, the rest remaining dormant. They occur opposite the median bundles of the sutbending leaves, not however, on the axis but on the leaves themselves and like the latter their insertion spread tangentially round the node (see De Bary, 1884, pp. 309–10; Majumdar, 1942, p. 50).

Text-Fig. 1 shows a transverse section of the apical bud with buds in different stages of development in the axils of primordia 5, 6 and 7. The first indication of bud initiation is noticed in relation to primordium 5 in a few cells of the adaxial epidermis opposite the median bundle (Figs. 2 and 3). The initiation is recognised by the vacuolating cells resuming active division and appearing more meristematic. Although the activity is level with leaf insertion it is distinctly confined to the primordial side of the union with *pr.* 4 in the outermost layer of which there is no suggestion of localised activity. Below the level of union the activity dies out soon (Fig. 4).

The next stage of bud development is seen in the axil of primordium 6 in the form of a pronounced lenticular mass of cells densely filled with protoplasm. The meristem continues up as a raised mass on the adaxial face

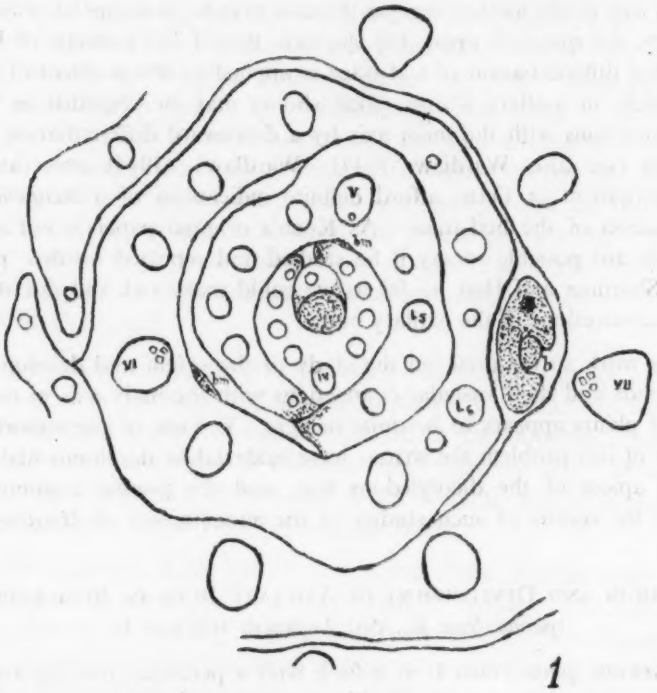
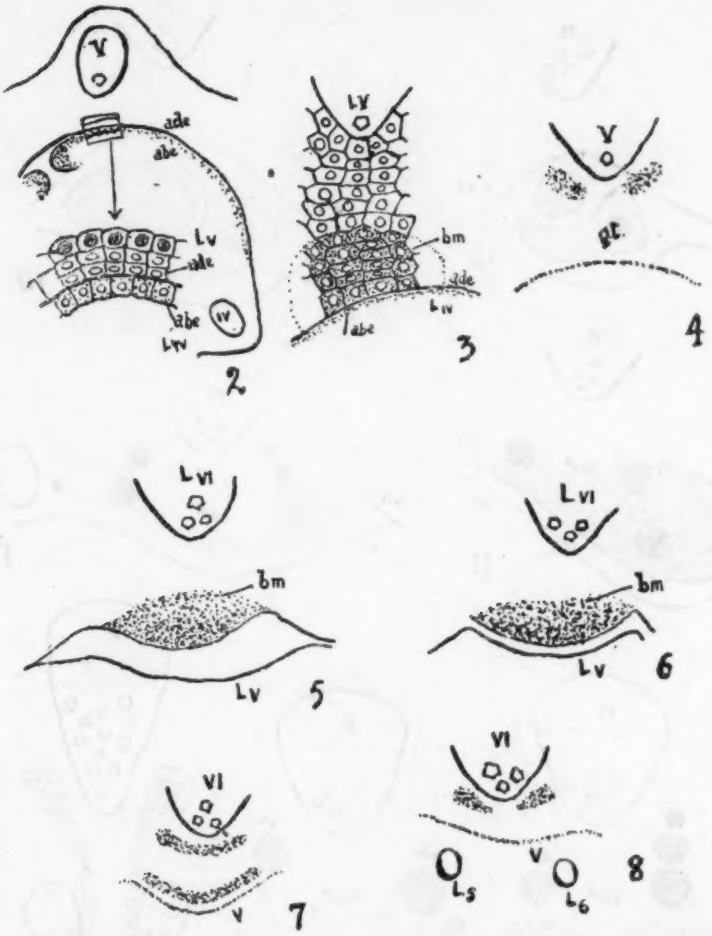


FIG. 1. T.S. of apical bud showing buds in the axils of primordia V, VI and VII in different stages of development; *M.* median bundle; *b.* axillary bud; *bm.* bud meristem; L_5 and L_6, laterals of pr. V and VI.

of the primordium, but it still dies out relatively rapidly in the downward direction and is lost in the vacuolating ground tissues of the *leaf-cushion*[2] just to the adaxial side of the median bundle (Figs. 5, 6, 7 and 8).

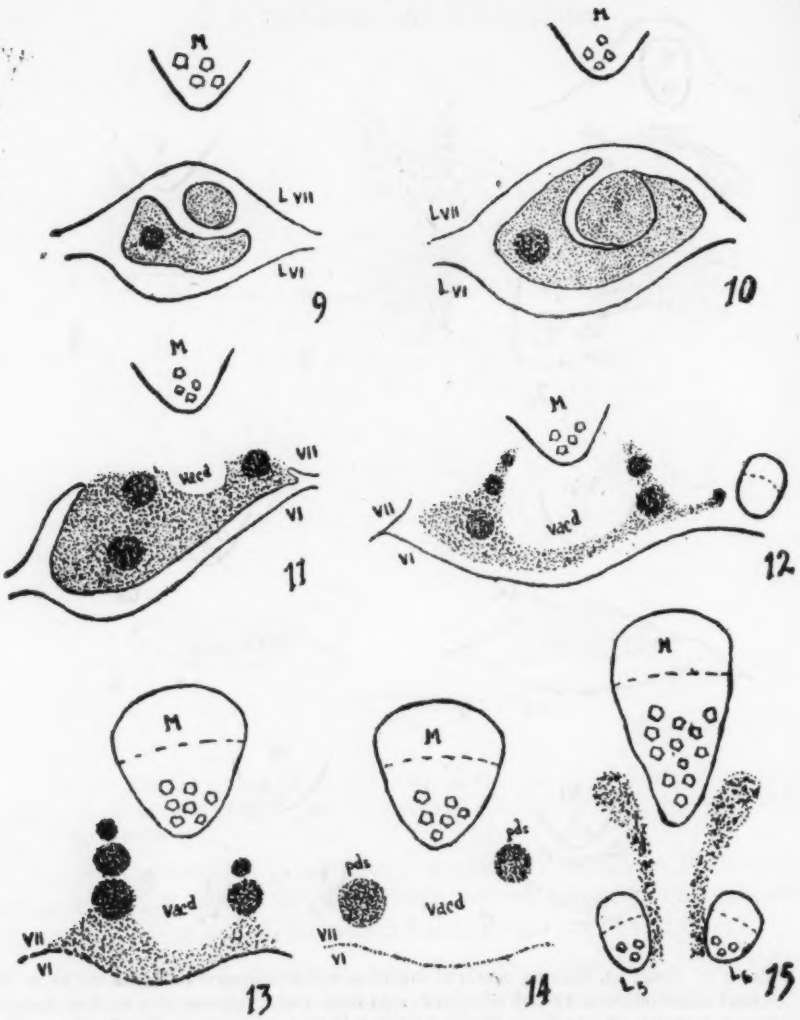
The next stage is seen in the axil of the 7th primordium. The bud axis now shows a central pith which is continuous outwards with the ground tissue of the primordium to either side of the median strand, but is enclosed to the adaxial side by an arc of meristem. The figure shows that the prodesmogen strands are differentiated from the marginal region of the arc, whilst their adaxial side is composed of more radially extended meristem cells which are also tending to spread beyond the arc along the adaxial side of the primordium at its level of union. Followed upwards this arc of meristem

[2] The term *leaf-cushion* is used to designate that region of the unit in which the leaf has joined the stem but the strands have not yet taken up their position n the vascular ring of the axis. This is actually equivalent to the region of the leaf insertion.

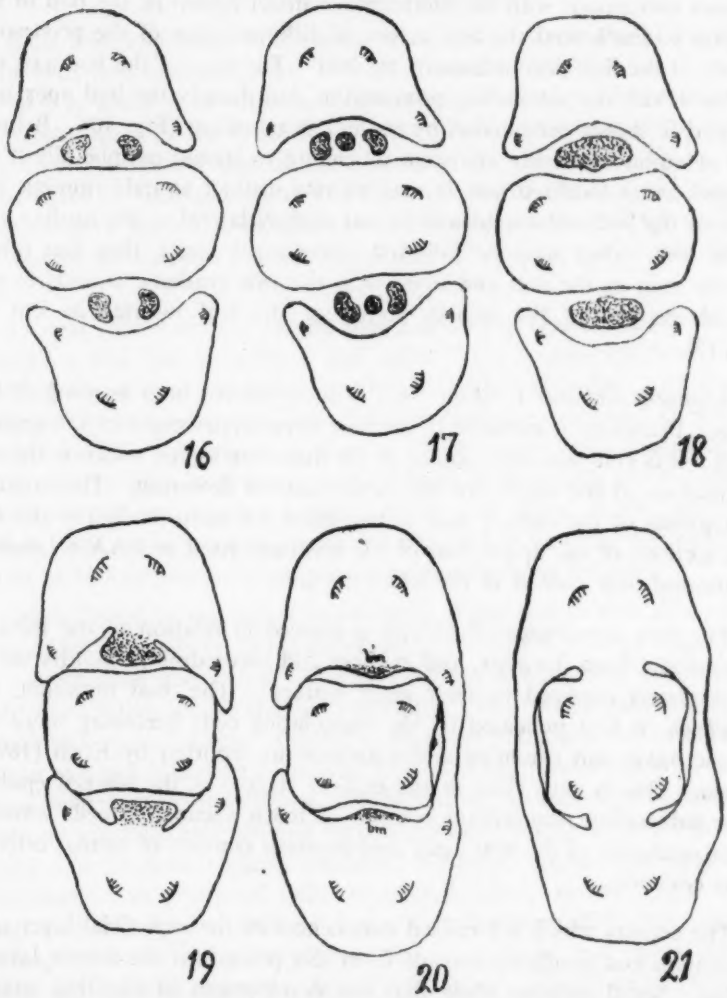


FIGS. 2–4. Serial t.s. showing details of initiation and development of bud in axil of pr. V. Fig. 2. Inset shows origin in the cells of adaxial epidermis (*ade*) opposite the median bundle; abaxial epidermal cells (*abe*) of pr. IV contain abundant starch grains. Fig. 3. Shows the lateral and abaxial spread of the bud meristem (*bm*). Fig. 4. At level of insertion on axis, shows bud meristem lost in the ground tissue (*gt*) of the leaf-cushion.

FIGS. 5–8. Serial t.s. of bud in the axil of pr. VI, description in the text; dotted line represents the line of union of bases of pr. V and VI in the axis.



FIGS. 9–15. Serial t.s. of bud in the axil of pr. VII; description in the text; *pds*, prodesmogen strands of the bud; *vacd*., vacuolated ground meristem. Fig. 12. Shows stage at level of insertion, L_5 and L_6 laterals of pr. V and VI, they join together to form a synthetic bundle in the axial ring (see Fig. 1).



FIGS. 16–21. *Leonurus sibiricus*. Serial t.s. of the shoot apex showing the foliar origin of the axillary buds. Fig. 21. Shows bud meristem, *bm*. lost in the ground tissue at the level of insertion of the primordia.

becomes continuous with the meristematic distal region of the bud in which the pith is visible and the first stages of differentiation of the prodesmogen strands of the first primordium of the bud. The base of the bud axis is still confluent with the subtending primordium, but distally the bud apex is now free and is shown surrounded by its first primordium (Fig. 10). Below the level of union where the meristem is tending to spread peripherally it again becomes more localised and is seen as two distinct strands running in the tissue of the leaf-cushion adaxial to and slightly lateral to the median strand of the leaf. They may be followed downwards until they run into the vascular ring in the axis and unite with the two synthetic strands to either side of the gap of the median strand of the leaf inserted at this node (Fig. 15).

Leonurus sibiricus L. (Figs. 16–21) is an annual herb growing in waste places. Branching is confined to the four to six upper nodes of the vegetative region; it is conspicuously absent in the flowering region which is the direct continuation of the vegetative one at the time of flowering. The origin and development of the axillary bud in this plant has been studied in the transverse sections of the apical bud of the seedlings fixed in FAA solution and microtomed and stained in the usual manner.

The first appearance of activity is noticed in relation to the third pair of primordia from the apex, and in these and succeeding primordia the activity is clearly confined to their inner surface. The bud initiation, as in *Heracleum*, is first indicated by the vacuolating cells becoming more meristematic again and resuming active division as reported by Koch (1893) in *Syringa*. This is very clear in the earliest stages, as the adaxial epidermis of the subtending primordium consists of much vacuolated cells whilst the abaxial epidermis of the next inner *soubassement* consists of similar cells with denser contents.

The activity which is localised commences in the superficial layer of the primordium and gradually extends from this point into the deeper layers of the leaf. Serial sections show that the development of the bud strand is basipetal, as it differentiates backwards into the axis to unite with the strands of the leaf outside the axial ring.

Thus the bud activity has the feature of being entirely *foliar in origin* and the vascular connection of the axillary bud is made with the bundles of the leaf-trace outside the vascular ring of the main axis in the leaf-cushion. This fact leads to the conformity of the foliar origin of the axillary bud.

Discussion and Conclusion

Buds normally occur in the axils of all shoots where the plant expands by an elaborate branch system. Very little work, however, has been done to follow in detail their origin and development. Such studies require careful investigations on the following points: (1) the time relationship between the origin of buds and their axillant leaves at the shoot apex, (2) the nature of the tissue or tissues concerned in the initiation of buds, (3) the positional relationship of the bud with reference to the axis and the leaf subtending the bud and (4) the vascular connections of the bud with the leaf and/or the main axis.

Goebel (1905) writing on the *time relationship* between the development of axillary bud and its axillant leaf states " the organs which are earliest unfolded are also earliest laid down " (ii, p. 432). Therefore, at the apex of the shoot the leaves are laid down first, the buds are developed later. As an example he cites the case of *Syringa* in which winter buds consist of leaves laid down one year in advance, and the buds in their axils are initiated only as these leaves unfold after a year. In all the cases so far reported the axillary buds are seen to initiate for the first time in the axils of second, third, fourth or fifth primordium, or pair of primordia from the apex.

With regard to the *nature of meristem* giving rise to an axillary bud reports differ, particularly, in the major groups of plants, such as Dicotyledons, Monocotyledons and Ferns. In Dicotyledons Koch (1893) reported origin in the vacuolating cells of the leaf primordia. The present studies (Majumdar and Datta) support Koch. Goebel's statement that the group of cells taking part in the bud initiation is derived from the embryonal tissue of the vegetative point is not very definite and precise as Schuēpp's (1926) *urmeristem* appears to include both *eu-* and *vacuolating* and *dividing meristem* of Priestley (1928). In Monocotyledons (Banana, *Triticum*, *Avena*, Maize and other Gramineæ), this group of cells belongs definitely to the *apical meristem* (Skutch, 1927; Rōsler, 1928; Kliem, 1937; Sharman, 1942, '45) situated on the opposite side of the insertion of the primordium just above the one in whose axil the bud appears. Hsü (1944) reports almost an identical origin in *Sinocalamus* sp. In Ferns the origin has been traced to the meristem which has been described by Wardlaw (1943) as the *detached* (apical) *meristem*. Study of a large number of shoot apices of Ferns, Gymnosperms, Dicotyledons and Monocotyledons is necessary before a general statement can be made on this question.

As regards the *positional relationship*, i.e., whether the origin is *foliar* or *axial*, near the base of the leaf or at other positions on axis or leaf, the

question has not been settled finally for which also a large number of shoot apices require to be investigated. In Dicotyledons Koch, Majumdar and Datta report *foliar origin* though Goebel makes it "from a group of cells of the axis of the shoot immediately above the insertion of a leaf", i.e., *axial*. Louis (1935) does not explain the confluent nature of the bud and its subtending leaf in *Ranunculus*. In Monocotyledons the position is definitely *axial* (Skutch, Rösler, Kliem, Sharman and Hsü), and the origin has been described as 'endogenous' by Hsü (1944). In Ferns Wardlaw (1943, '44) noticed the axial origin of the bud. According to him even the petiolar and extra-axillary buds in Ferns are axillary in origin, the final position being due to the growth adjustments between the axis and the leaf.

Reports so far made are unanimous on the mode of *vascular connections* of the axillary bud with the axillant leaf and the main axis. *Bud trace* originates in the primordium of the bud and then differentiates backwards into the leaf-cushion and finally joins the leaf-trace bundles in the vascular ring of the main axis. The differentiation of the bud strand is, therefore, *basipetal*.

Bud initiation appears to be essentially related to some physiological processes, as it always takes place in the axils of primordia which are removed some distance from the growing apex, and are in an active state of growth and differentiation. This is supported by the fact that in *Syringa*, where the leaves of the winter buds are laid down in the preceding year, the buds in their axils are only laid down as they unfold after a year.

Summary

1. Detailed studies on the initiation and development of axillary buds in *Heracleum* and *Leonurus* have been reported in this paper.

2. Works so far done on this problem in Ferns, Monocotyledons and Dicotyledons have been briefly reviewed.

3. In Ferns an axillary bud is initiated in the *detached* (apical) *meristem* on the free surface of the axis or "in proximity to meristele conjunctions". In Monocotyledons initiation takes place in the surface layer of the *corpus*, on the side opposite to the insertion of, and in association with, the leaf primordium just above the one in whose axil the bud appears. Hence Hsü (1944) describes the origin as 'endogenous' in Monocotyledons. In Dicotyledons though Goebel reports origin from the embryonal meristem a little behind the apex, Koch, Majumdar and Datta report origin in the vacuolating cells of the adaxial epidermis of the subtending leaf opposite the median bundle in *Syringa*, *Heracleum* and *Leonurus*.

4. In the shoot apices of Ferns, Monocotyledons and Dicotyledons leaf primordia are laid down first and buds are initiated in their axils later. A year may elapse between the laying down of the axillant leaves and formation of buds in their axils as reported in the winter buds of *Syringa*.

5. Bud trace originates in the bud primordium and then differentiates backwards into the *leaf-cushion* outside the axial ring of vascular bundles, finally it enters the ring to unite with one of its synthetic bundles.

6. As the buds normally originate in the axils of leaves which are removed some distance from the apex and which are growing vigorously or unfolding, it is suggested that their initiation is due to some physiological processes than to any specific qualities inherent in the apical meristem.

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CHÆMOTROPIC RESPONSE OF FRUIT FLIES

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Introductory

Fruit flies are the pests of great economic importance since they cause considerable damage to most of our fruits in the gardens. In some districts of this province valuable fruit crops such as guava, mosambi and mangoes and vegetables as cucurbits are severely infested and the damage may extend to about 40% in some cases. With a view to finding out some effective methods of control, investigations were taken up at Poona which were in progress since 1937. Experimental evidence based on control operations carried out all over the world by various workers, namely, Jarvis (1931) and Newman (1935) from Australia, Bua (1934) and Silvestri (1934) from Italy, Isaskides (1945) from Greece, Ripley and Hepburn (1935) from Africa, Chopra (1926-27) and Hussain (1927-28) from India, proved that on the whole, trapping these insects with the help of ammoniated material, poison baits or the destruction of affected fruits were the most effective remedies in the control of fruit flies. Our investigations were also carried out practically on the same line and the data pertaining to trapping experiments is presented below.

To start with, Clensel 'A', Clensel 'B', ammonia vanilla and citronella oil were tried as attractants. Citronella oil attracted the males only hence it was discontinued for further trials. Out of Clensel 'A', Clensel 'B' and ammonia vanilla, Clensel 'A' yielded relatively higher catch throughout the year hence further trials were restricted to that material only. The material was obtained from Clensel works, Howrah, Calcutta and the trials were carried out at the Government fruit experimental Station, Kirkee. Our thanks are due to Mr. S. R. Gandhi, the Superintendent of the garden, for his help and willing co-operation during the course of these investigations and to the Imperial Entomologist, New Delhi, for identifiying the specimens for us.

Technique

Clensel diluted in the ratio of 1:20 was put in specially constructed trap bottles which were hung on the trees and distributed all over the plot with a distance of 30–35 ft. between them on all the sides. Observations were made every week and the contents in the traps renewed. During the first two years the traps were allowed to remain in the plots throughout the year but later on since the collections were obtained only during the fruiting season, the traps were removed after the fruits were harvested.

Preliminary observations were made with glass traps prepared from ordinary wide-mouthed bottles. These did not prove satisfactory since the contents evaporated within a week's time, hence these were replaced by specially prepared traps. Ordinary 1 lb. jam jars with a small circular hole cut in the lid for entrance of the flies were used with a special hooked arrangement attached to them for hanging. These proved very satisfactory and yielded good results. During these investigations various strengths of Clensel were tried but 1:30 ratio yielded very encouraging results (Table I), which is in confirmity with Newman (1935).

Table I

Relative collection of fruit flies under different strengths of Clensel dilutions

Date	1 : 10	1 : 20	1 : 30	1 : 40	Average percentage of attraction with different dilutions
1938					
1—V	..	4	6	3	
11—V	..	10	63	17	
19—V	..	33	37	21	
26—V	..	36	94	31	
2—VI	..	442	269	98	
9—VI	..	80	230	56	
15—VI	3	22	11	12	
26—VI	..	..	3	1	1 : 10— 0·2
3—VII	..	3	1	..	1 : 20—39·8
10—VII	..	1	..	..	1 : 30—44·9
24—VII	..	..	..	..	1 : 40—15·0
7—VIII	..	..	..	..	

Since 1:30 dilution yielded comparatively better results this strength was given extensive trials in later experiments. Efforts were also made to test the combined efficacy of Clensel and citronella oil, but as it was found that only the males were attracted, it was not tried any further.

Collections

In cucurbit fields practically all the species of the fruit flies were collected but *D. cucurbitæ* predominated in the collection being 90·5% against 9·5% *D. zonatus*. In the case of guava, *Mosambi* and mango, however, *D. ferrugineus* was the predominating species. The seasonal activities of all the species showed that on the whole, the collection was appreciably higher during April to July being about 60% of the total catch whereas during other months it was relatively low (Table II).

The seasonal fluctuations were further correlated with the atmospheric temperature and it was noticed that the rise in the collection practically corresponded with the rise in the average minimum temperature (Text-fig. 1). Although, it has been observed that clensel attracts the males in relatively larger numbers, the differences however, were not very marked in the case of the predominating species namely *D. ferrugineus*, *D. zonatus* and *D. cucurbitæ*.

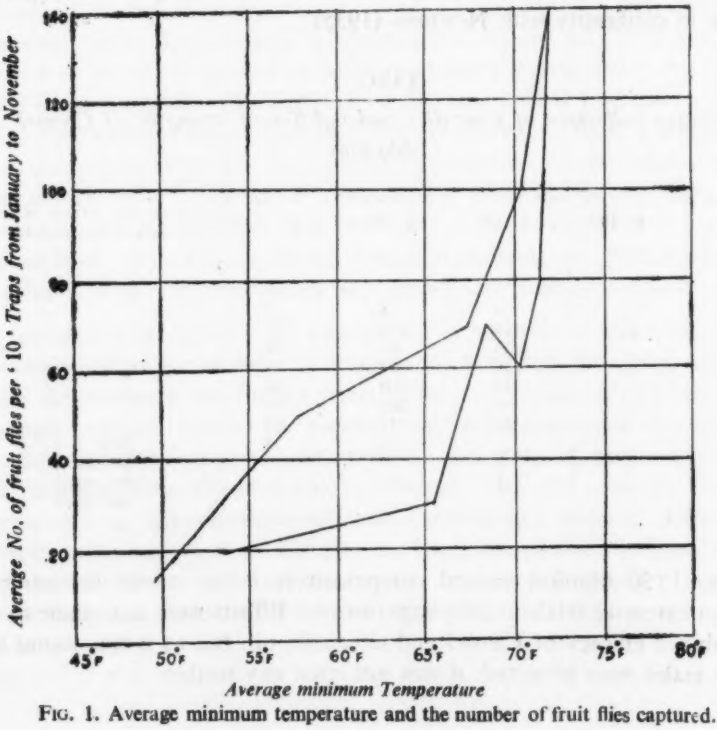

Fig. 1. Average minimum temperature and the number of fruit flies captured.

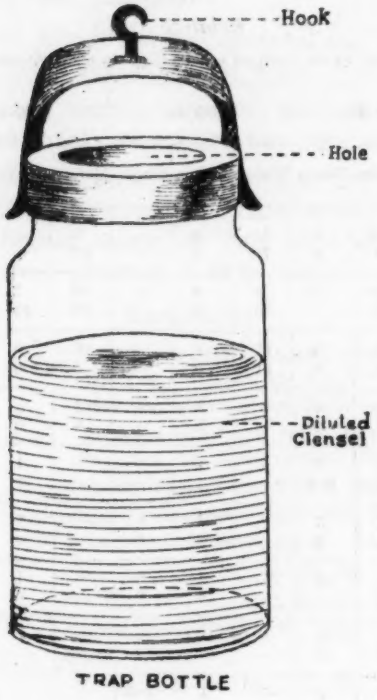

FIG. 2

EFFICACY

Trapping operations with clensel were started with a view to checking the damage by the fruit flies. This however, was not materialised since during the course of our observations, clensel alone or in combination with other materials did not help appreciably in reducing the damage since the control in the treated plots by trapping was not, on an average, more than 5% over the untreated ones. This was specially noticed in the case of guava plots, where in spite of high infestation and correspondingly heavy damage ranging between 30–40%, there was practically no collection of flies in the traps. Further observations in this respect were made by reducing the distance between the traps from 30 feet to 15 feet on all sides. In other words, practically one trap was used per tree. This yielded slightly better result but the cost was raised considerably which almost neutralized the beneficial effects of the traps.

TABLE II

Relative catch

No. of flies caught per 10 traps under different crops

	1938–39			1939–40			1940–41			1941–42		
	Mango	Citrus	Guava	Mango	Citrus	Guava	Mango	Citrus	Guava	Mango	Citrus	Guava
April ..	9	4	0	12	8	—	8	—	—	22	—	—
	11	1	0	18	2	—	12	—	—	10·5	—	—
May ..	50	0	0	20	0	—	15	2	—	5	—	—
	55	0	0	30	0	—	10	10	—	8	—	—
June ..	15	0	0	19	4	—	8	0	—	80	—	—
	7	0	0	11	0	—	5	0	—	30	—	—
July ..	6	3	0	—	7	—	4	0	—	10	—	—
	1	2	0	—	3	—	0	0	—	8	—	—
August ..	0	7	0	—	2	0	—	1	1·6	—	—	1·6
	0	5	0·6	—	3	2·5	—	1	0	—	—	—
September ..	0	1	3	—	1	1	—	0	0	—	—	—
	0	1	0	—	0	0	—	4	4	—	—	—
October ..	0	0	6	—	1	0	—	1	1	—	2	1
	0	1	1	—	0	0	—	0	0	—	—	3
November ..	0	1	5	—	0	5	—	—	2	—	0	3
	0	1	0	—	2·5	10	—	—	0	—	2	2
December ..	0	1	5	—	0	2·5	—	—	2	—	—	6
	0	1	2	—	0	0	—	—	4	—	—	6
January ..	0	2	3	—	1	0	—	—	3	—	—	—
	0	0	0	—	2	4	—	—	0	—	—	—
February ..	8	1	1	—	1	0	—	—	—	—	—	—
	16	0	4	—	2	0	—	—	—	—	—	—
March ..	1	7	0	—	7	0	—	—	0	3	—	—
	8	3	0	—	1	0	—	—	—	4	—	—

— Indicates 'No traps kept'.

0 Indicates 'No collection'.

N.B.—Under each month figures are given for each fortnight.

Clensel traps were also tried along with tarter emetic bait on an extensive area but the results were in no way better and the cost is even prohibitive.

SUMMARY

The fruit flies show chæmotropic response. Citronella oil, ammonia vanilla and clensel were tried but none of them yielded satisfactory results. Clensel, however, had a slight advantage over others.

Clensel when tried in 1 : 30 dilution gave higher percentage of the attraction as compared with other dilutions.

On an average, *D. ferrugineus* predominated over other species in the collection during the entire period of four years.

Males were captured slightly more in numbers but the differences between the males and females were in no way significant.

Clensel even when tried on a very large scale did not help in reducing the damage by fruit flies to any appreciable extent and much less economically. For economy trap bottles should be kept at a distance of 30 ft. even though better results were obtained at 15 ft.

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BREEDING BRINJALS (*SOALNUM MELONGENA*) IN MADRAS

I. Hybrid Vigour in Brinjals*

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(Communicated by Dr. T. S. Sadasivan, M.Sc., Ph.D., F.A.Sc.)

The vital phenomenon of hybrid vigour in the brinjal has been studied by a number of workers, but all outside India. Pal and Singh (1943) are believed to be carrying on some studies on the practical utilization of this phenomenon and as yet their results are not made available. Considerable work on brinjal hybridization is reported to have been attempted at the New Jersey Agricultural College Experimental Station (Bayla, 1918), and interesting work on these lines has also been carried out in Japan. Bayla (1918) concluded that the F_1 hybrid plants were very much more vigorous, strong and healthy than either parent. Increase in yield in the hybrids has been recorded by many workers (Nagai and Kida, 1926; Tatesi, 1927; and Kakizaki, 1931, in Japan; Aver'Janova, 1941; Daskaloff, 1941, in Bulgaria). The cross that showed the highest increase yielded 140·8 per cent., more than the better parent, while another cross registered an increase of 222·2 per cent., over the poorer parent (Kakizaki, 1931).

Kakizaki (1931) came to the conclusion that the degree of hastening in fruit production of brinjal crosses is widely different according to the different combinations of varieties, that the cross is earlier than the average of both the parents in a majority of the cases, and that often the cross is earlier than the early parent. Schmidt (1935) reported similar results and observed that earliness was dominant and even transgressive and in one cross it even exceeded the earliest variety. He also observed that the combinations comprising the earliest variety were extremely early. Aver'Janova (1941) obtained similar results.

The possibility of capitalising hybrid vigour depends upon its extent and the cost of producing hybrid seed and it is likely to be most attractive commercially when a little labour results in the production of a large amount of seed (Hadfield and Calder, 1936). The brinjal is an example in which one cross will furnish a large amount of seed and it has been found by Kakizaki (1930) that the measure of improvement resulting from certain crosses more than compensates for the cost involved in producing the hybrid

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seed. Daskaloff (1941) agrees with Kakizaki and remarks that it is perfectly possible to produce heterosis or hybrid varieties on a commercial basis. It will be interesting to note that the Saitama Agricultural Experimental Station in Japan was yearly furnishing gardeners with F_1 seeds of two crosses of an amount to be used for the commercial culture of a total of about one hundred acres (Kakizaki, 1930).

With a wide collection of brinjal varieties in our country it is deemed possible that an improvement of this vegetable crop can be effected through the employment of F_1 hybrids, and as a preliminary step towards an intensive breeding project, the study of hybrid vigour in some Indian brinjals was undertaken by the author at the Agricultural Research Institute, Coimbatore, during the years 1943 to 1945.

Materials and Methods

Materials

Seeds of all the available varieties of this vegetable were collected from various sources both from within and outside the province of Madras. A detailed study of all the samples was made and such of those with fixed characters were isolated for further study. For the present study two varieties, *viz.*, "Raviya" from Baroda, and "Udipi" from the College Orchards, Coimbatore, were selected. These varieties differ from each other in many respects. The fruit of the Raviya is round in shape and dark purple in colour, while that of the Udipi resembles that of the former in size and shape, but it is dusky green in colour with white streaks extending upwards from the stigmatic end. While the Raviya is thornless, the Udipi is beset with thorns.

Methods

Emasculation.—The brinjal flower is in nature cross-pollinated by insects. The flowers are well adapted for this kind of pollination. Hence prior to the crossing of the flower there is a need for emasculation and bagging so as to avoid any chance contamination by insect or other agents. The emasculation is easy in the case of this plant and the method followed is the same as that described by Kakizaki (1931).

Crossings made.—For the study of hybrid vigour in the first generation the following crosses were made:—

1. Udipi as the pistillate or female parent and Raviya as the staminate or male parent, and

2. Raviya as the pistillate parent and Udipi as the staminate or male parent.

The seeds of the crossed fruit were collected when they were mature. To study the F_1 generation in comparison with the parental strains seeds were collected from the selfed fruit of both the parents also. The number of seeds in both the crossed and selfed fruit were counted and recorded. The weight of the seeds of the crossed as well as the selfed parental fruit was also determined.

The hybrid and the parental seeds were sown in equal numbers in seed pans and the germination percentage and the performance of the seedlings were observed in comparison with one another. The seedlings were transplanted in the field when they were a month old. The cross Udipi ♀ × Raviya ♂ was studied in detail in respect to all the plant characters. The results obtained are embodied in a subsequent part of this paper.

Experimental Results

1. *The percentage of seediness of the crossed fruit as compared with that of the parental ones.—*

TABLE I. *Showing the weight of the crossed fruit, percentage of seediness and the number of seeds as compared with those of the selfed fruits of the parents*

No.	Sample or cross	Weight of fruit in Gms.	Weight of seeds in Gms.	% seediness	No. of seeds in a fruit
1	Udipi ..	113·3	8·5	7·5	2350
2	Raviya ..	117·4	6·34	5·4	1915
3	Udipi ♀ × Raviya ♂ ..	102·3	7·06	6·9	1800
4	Raviya ♀ × Udipi ♂ ..	93·0	5·60	6·02	1568

The percentage of seediness of the crossed fruit is intermediate between that of the parent fruit. The number of seeds in the crossed fruit is less than that in the fruit of both the parental strains.

2. *Weight of seeds.—*

TABLE II. *Showing the weight of 500 crossed seeds as compared with that of the parent seeds*

	Particulars	Raviya ♀ × Udipi ♂	Udipi ♀ × Raviya ♂
1	Weight of 500 seeds of the cross ..	1·81 gm.	1·96 gm.
2	,, ,, of female parent ..	1·67 ,,	1·74 ,,
3	,, ,, of male ..	1·74 ,,	1·67 ,,
4	,, ,, of average of parents ..	1·71 ,,	1·71 ,,
5	% increase of the cross over—		
	(*a*) Female parent ..	8·38	12·64
	(*b*) Male ,, ..	4·02	17·36
	(*c*) Average of parent ..	5·57	14·95

The crossed seeds are heavier than the parent seeds in both the crosses. They are also found to be heavier than the average of both the parents.

TABLE III. *Showing the weight of the embryo and the seed-coat of* 500 *seeds of the crosses as compared with those of the parents*

Particulars	Raviya ♀ × Udipi ♂	Udipi ♀ × Raviya ♂
1 Weight of 500 seeds of the cross ..	1·81 gm.	1·96
2 Cross—		
(*a*) Weight of seed-coat ..	0·36 ,,	0·39
(*b*) ,, embryo ..	1·45 ,,	1·57
3 Female parent—		
(*a*) Weight of seed-coat ..	0·34 ,,	0·36
(*b*) ,, embryo ..	1·33 ,,	1·38
4 Male parent—		
(*a*) Weight of seed-coat ..	0·36 ,,	0·34
(*b*) ,, embryo ..	1·38 ,,	1·33
5 Percentage increase of weight of embryo over		
(*a*) Male parent ..	5·07	13·71
(*b*) Female parent ..	9·02	18·04

The increase in weight of the crossed seed is due to the increase in weight of the embryo portion of the seed. The weight of seed-coat is almost the same as that of the heavier parent. The embryo of the crossed seed is distinctly heavier than that of both the parents.

3. *Germination capacity.*—

To determine the germination capacity of the hybrid seeds as compared with those of the parents six experiments were carried out and the average percentage of germination of the parents, Udipi and Raviya, and of the hybrids Raviya ♀ × Udipi ♂ and Udipi ♀ × Raviya ♂ were 66·1, 69·3, 71·6 and 71·0 respectively. From the data obtained it appears that the percentage of germination of the crossed seeds is greater than those of the seeds of the selfed parental strains, and that possibly the hybrid vigour is also manifest in the better germination of the hybrid seed.

4. *Comparative height when* 50 *days old.*—

TABLE IV. *Showing the average height of the* F_1 *plants in comparison with that of the parent plants at the time of transplanting* (50 *days old*)

Name of sample or cross	Average height of plant in each set of 20 plants in set No.					Average height of the plant in inches
	1	2	3	4	5	
1 Udipi ..	4·00	3·76	3·50	3·87	4·10	3·75
2 Raviya ..	3·42	3·80	4·20	3·25	3·76	3·67
3 Udipi ♀ × Raviya ♂	3·70	3·95	4·23	4·00	3·35	3·85

The difference between the height of the F_1 seedlings and that of the male and female parent seedlings is not appreciable.

4. *Plant characters.*—

TABLE V. *Showing the plant characters of the F_1 plants and the parental plants (average)*

Plant characters	Udipi	Udipi ♀ × Raviya ♂	Raviya
1 Height in cm. ..	62·0	65·5	54·0
2 Spread in cm. ..	56·5	85·0	82·0
3 Colour of stem and prickliness	All parts green, thorny, thorns green	Tender parts slightly purple, no thorns	Tender parts purple, no thorns
4 Leaf length in cm. ..	9·3	12·9	13·5
5 Leaf width in cm. ..	6·2	7·2	8·7
6 Margins of lamina ..	Slightly wavy	Slightly wavy	Slightly wavy
7 Inflorescence ..	Compound type	Compound type	Compound type
8 Flower colour ..	Light violet	Violet	Deep violet
9 No. of days to flower from date of sowing	125	107	128
10 Fruit colour ..	Dusky green with white streaks from apical end	Dark purple and shiny	Dark purple and shiny
11 Fruit shape and size ..	Round and medium	Round and medium	Round and medium
12 No. of fruits per plant ..	10	8	7
13 Yield in weight per plant in ozs.	24·0	42·2	36·0
14 % fruit attacked by fruit borer	12·0	17·2	17·6
15 Bud worm infection ..	Found	Found	Found

From the foregoing it is observed that—

(1) The F_1 plants are taller than those of both the parents and this hybrid almost resembles the female parent (Plate No. IX).

(2) In spread also the F_1 plants surpass both the parents.

(3) The F_1 plants are thornless like the male parent, and also resemble it in respect to the stem colouration.

(4) In the leaf character it is intermediate between the parents.

(5) In the colouration of the flowers, *viz.*, corolla, it is intermediate between the parents.

(6) In flowering, the hybrid plants are early flowering and they are 18 days earlier than the early parent.

(7) The purple colour of the fruit of the male parent is found to be dominant over the green mottled with white of the female parent.

(8) The fruit of the hybrid is of the same size and shape of both the parents.

(9) With regard to the number of fruit produced per plant the hybrids are intermediate between the parents.

(10) In respect to yield per plant by weight, the F_1s excel both the parents, the percentage of increase over that of the male and female parents being 24·1 per cent., and 17·2 per cent., respectively.

(11) With regard to the resistance or susceptibility to insect pests such as the fruit borer and the bud worm, the hybrid plants did not show any appreciable improvement over those of the parents.

Discussion

It has already been explained by a number of workers that appreciable increase in yield through the employment of F_1 hybrids is possible in the brinjal (Aver'Janova, 1941; Daskaloff, 1941; Kakizaki, 1931; Nagai and Kida, 1926; Tatesi, 1927). The practical utilization of this most promising line of improvement is partially limited firstly by the wide variation in the extent of improvement between the F_1 progenies of different intervarietal crosses ranging from 1 to 222 per cent., in the studies of Kakizaki (1931), and 17·2 per cent., over the better parent and 24·1 per cent., over the poorer parents in the present studies, and partly by the failure of some intervarietal crosses to register any improvement (Kakizaki, 1931). The possibility of decreased yield through such intervarietal crosses though confined to only a very few cases is sufficient to warrant the necessity for detailed investigation with the object of determining the optimum parental combinations. The possibility of capitalising hybrid vigour also depends upon the economics of such seed production. Fortunately crossing in brinjal has been found easy in the present study as well as by other workers, and each crossed fruit contains a very large amount of seed. Since the measure of improvement resulting from such crosses more than compensates for the cost involved in producing the hybrid seed, it is quite possible to produce hybrid varieties on a commercial basis.

It has been found that the F_1 plants of the cross between Udipi ♀ and Raviya ♂ has given clear indication of earlier germination and earliness of crop maturity than both the parents; earliness of flowering is a good varietal trait particularly from the point of view of the market. In yield the average of the hybrid plants has been recorded at 42·2 ounces per plant as against the mean yield of 34·0 and 36·0 ounces in the male and female plants respectively. The other promising feature in the F_1 is that it possesses some of the qualities desirable of a good brinjal plant, *viz.*, (1) tallness, (2) good lateral expansion and branching habit and (3) fruit of attractive

colour and decent shape, soft and with less seeds and the absence of thorns. All these represent economic improvements which indicate in some measure what can be expected from the pursuit of research on more extended lines.

Thus these preliminary observations on the influence of crossing on the performance of the first generation hybrid plants are suggestive of the potentialities. These various observations provide the necessary background for bringing about effective improvement through the use of hybrid vigour.

Summary

1. For the study of hybrid vigour two varieties differing very widely from each other were chosen. In the cross between the varieties Udipi as the female parent and Raviya as the male parent, it has been observed at the very outset that the immediate effect of crossing manifest itself in the increased weight of the F_1 seeds. The F_1 seeds had a slightly higher percentage of germination than that of the parental strains.

2. The F_1 seedlings and plants exhibited better vigour than the parental seedlings from the very start. The cross resembled the male parent with respect to the fruit characters.

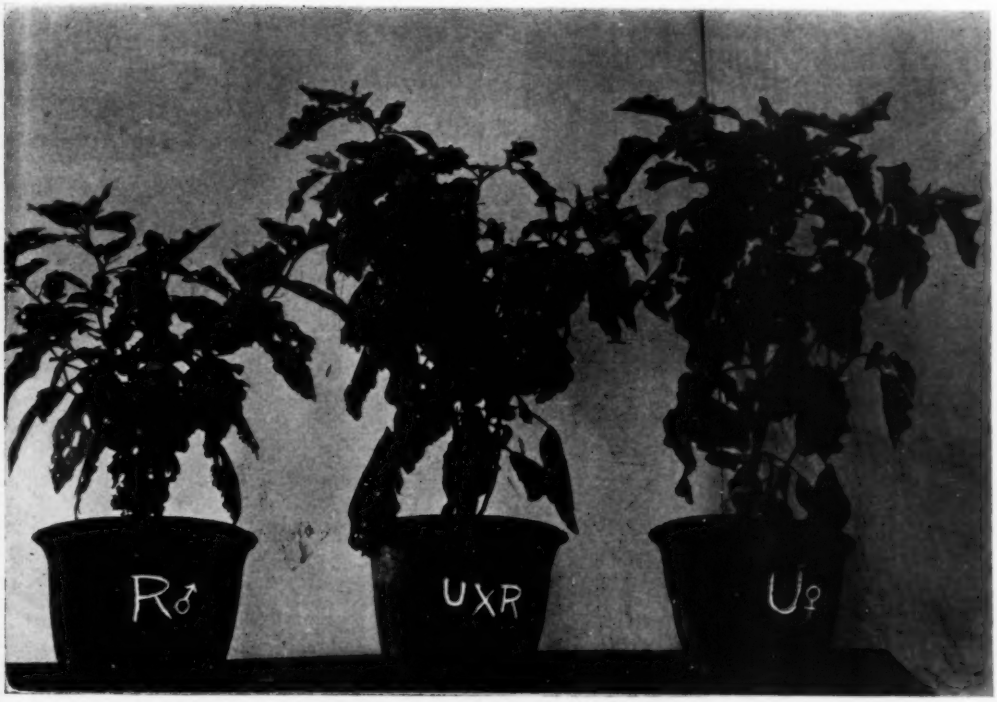
3. The thorny character of the female parent was totally absent in the case of the hybrid plants.

4. The hybrids flowered earlier than the earlier parent by 18 days, and yielded more than both the parents, the increase in yield being 17·2 per cent., over the better parent and 24·1 per cent., over that of the poorer parent.

5. Other observations with regard to plant and fruit characters are detailed in the text.

6. The importance of hybrid vigour in the brinjal crop improvement is therefore indicated.

In conclusion, the author wishes to gratefully acknowledge the kind help he had received from various sources during the course of his study. He is greatly indebted to his Professor, Sri. K. C. Naik, the Fruit Specialist to the Government of Madras, who suggested the problem and gave all necessary encouragement during the course of the study.



Showing the parents and the F_1 hybrid

R ♂—Raviya variety (male parent)
U ♀—Udipi variety (female parent)
U ×R—The F_1 cross between Udipi ♀ and Raviya ♂

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A CONTRIBUTION TO THE LIFE-HISTORY OF *SPHENOCLEA ZEYLANICA* GAERTN.

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The earlier literature relating to the embryological features of the Campanulaceæ has been reviewed by Schnarf (1931), and the recent work has been described by Kausik and Subramanyam (1945) in their paper on the embryological study of *Isotoma longiflora* Presl. *Sphenoclea zeylanica* Gaertn., a monotypic species belonging to this family, has been selected for the present investigation. It is found in moist districts, in swampy places, especially near the coast. It is a stout herb reaching one to three feet in height with oblong-lanceolate, glaucous green leaves. The greenish-yellow flowers are found in terminal close spikes. Fruit is a membraneous depressed globose-capsule with the upper portion of the capsule opening out in the form of a lid.

Material and Methods

The material for the present study was collected at Guddancherry, a suburb of Madras. It was fixed in formalin acetic alcohol and Allen's modified Bouin. At 70% stage in dehydration the ovaries were placed in Carnoy's fluid for half an hour to facilitate slight hardening of the material and thus preventing the detachment of the ovules from the placenta. Sections were cut ten to twenty μ in thickness and stained in Heidenhain's iron-alum hæmatoxylin with eosine as counterstain for contrast.

Microsporangium

The wall of the young anther (Fig. 1) shows three layers below the epidermis ; of these the outer is the endothecium and the inner the tapetum, with a single middle layer in between. The endothecium shows fibrillar thickenings when the anther is mature, while the middle layer disorganises, and the tapetum, which is uninucleate to start with, becomes binucleate later. At the same time, the tapetal cells are also conspicuously vacuolate (Fig. 1). The microspore mother cells undergo the usual reduction divisions and form the tetrads of microspores. The separation of the microspores is brought about by cleavage furrows (Fig. 3) which start at the periphery. The pollen grains are arranged in a tetrahedral, or sometimes, in an isobilateral manner (Fig. 2). The mature pollen grain is trinucleate (Fig. 4), with a thick rigid

outer exine and a thin delicate intine. Three germ pores are seen in the wall of the pollen grain.

Organogeny of the Flower

In the organogeny of the flower the different floral parts take their origin in an acropetal succession, the order being, the sepals, the petals, the stamens and finally the carpels.

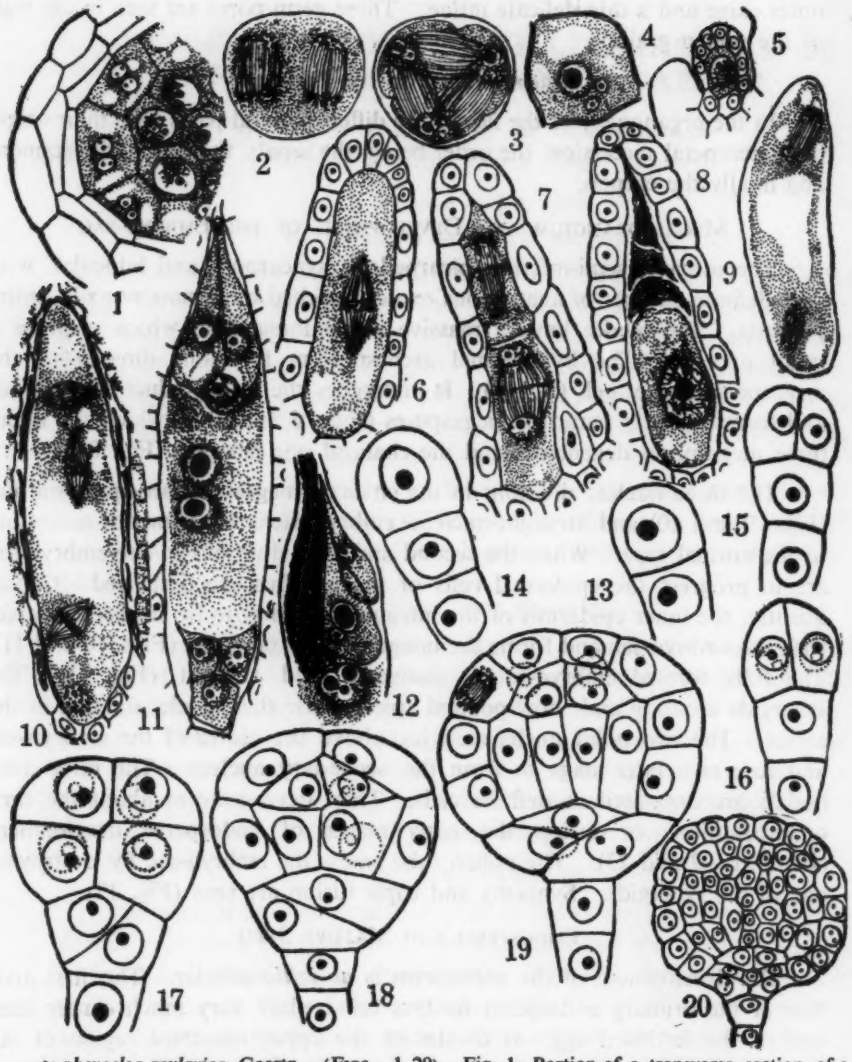
Megasporangium and Development of the Embryo-sac

The ovary is semi-inferior, bicarpellary, syncarpous and bilocular, with an indefinite number of anatropous ovules attached on a massive axile entire placenta. The ovules have a massive single integument which encloses a small nucellus. The hypodermal archesporium functions directly as the megaspore mother cell (Fig. 5). It undergoes the usual reduction divisions and forms a linear tetrad of megaspores (Figs. 6 and 7) of which the upper three megaspores degenerate and the chalazal one enlarges (Fig. 8).

The three nuclear divisions in the chalazal megaspore are quite normal (Figs. 9 and 10) and an eight-nucleate embryo-sac is thus formed according to the normal type. When the second and third divisions in the embryo-sac are in progress the epidermal cells of the nucellus are destroyed. Consequently, the inner epidermis of the integument comes to lie in direct contact with the embryo-sac and forms the integumentary tapetum (Figs. 10 and 11). The fully formed embryo-sac is elongated and straight (Fig. 11). The synergids are long and show pointed apices, with their nuclei situated in the centre. The two polar nuclei meet just above the centre of the embryo-sac and fuse at a later stage to form the secondary nucleus. The three antipodals are organised as definite cells. They degenerate at about the time of fertilisation, or during the early stages of endosperm development (Figs. 21, 22 and 23). The pollen tube enters the embryo-sac by destroying one of the synergids. Syngamy and triple fusion are seen (Fig. 12).

Endosperm and Mature Seed

The development of the endosperm is *ab initio* cellular. The first division of the primary endosperm nucleus takes place very much earlier than that of the fertilised egg. It divides in the upper one-third region of the embryo-sac, and is followed by a transverse wall to form a small upper primary micropylar chamber and a large lower primary chalazal chamber (Fig. 21). The next division then follows in both these chambers by means of vertical walls, that in the upper being earlier than the one in the lower (Figs. 22 and 23). Sometimes, however, the chalazal chamber shows the formation of a transverse wall, instead of a vertical one (Fig. 24). After the four-celled



Typhonodora zeylanica Gaertn. (FIGS. 1–20). Fig. 1. Portion of a transverse section of a young anther showing wall layers, binucleate tapetum and microspore mother cells. × 1260. Figs. 2 & 3. Second division in microspore tetrad formation. × 2700 each. Fig. 4. A mature trinucleate pollen grain. × 2700. Fig. 5. Megaspore mother cell. × 900. Fig. 6. Metaphase division in the megaspore mother cell. × 1800. Fig. 7. Second division in the formation of the linear tetrad. ×1800. Fig. 8. Enlarging chalazal megaspore and the degenerating upper three megaspores. × 1800. Figs. 9 & 10. Second and third nuclear divisions in the formation of the embryo-sac; note the degenerating nucellar epidermis and the formation of the integument

tapetum. × 1800 each. Fig. 11. Fully organised embryo-sac showing the egg apparatus, antipodal cells and the two polar nuclei. × 1800. Fig. 12. A stage in double fertilisation showing remnants of the pollen tube. × 1800. Fig. 13 Two-celled proembryo. × 1800. Fig. 14. Three-celled proembryo. × 1800. Fig. 15–17. Stages in the formation of the octant stage of the embryo. × 1800 each. Fig. 18. The separation of dermatogen by periclinal walls. × 1800. Figs. 19 & 20. Later embryos showing the differentiation into dermatogen, periblem and plerome. In Fig. 19 note the formation of hypophysis by two interesting oblique walls. Fig. 19 × 1800, Fig. 20. × 900. (Original magnifications are indicated here, but the figures have been reduced to two thirds in reproduction).

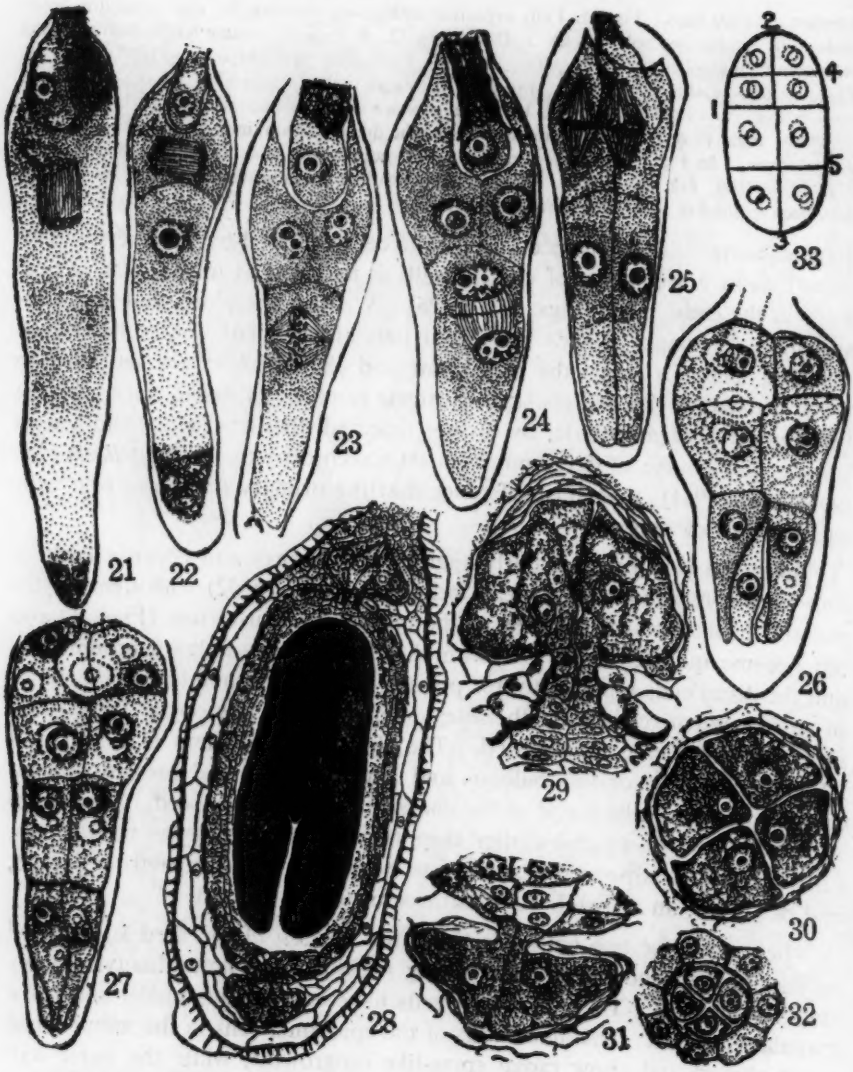
stage subsequent divisions occur in all the cells followed by transverse walls, as well as by a second set of vertical walls at right angles to the first vertical walls in the embryo-sac (Figs. 25 and 26). We find, therefore, that the endosperm, at this stage, is made up of four tiers of cells, with four cells in each tier (Fig. 27). Of these, the micropylar and chalazal tiers are set apart to form the micropylar and chalazal haustoria respectively, while the remaining two central tiers contribute, by further repeated divisions, to the endosperm tissue. The course of development is thus according to the *Scutellaria*-type of Schnarf (1931), with this difference that the primary tiers have four cells each instead of only two.

Both the haustoria are fairly aggressive structures and consists each of four very conspicuous uninucleate cells (Figs. 30 and 32) with dense cytoplasm. In later stages the cells of the micropylar haustorium (Figs. 29 and 30) become quite characteristic with their outer sides bulging prominently and the distal ends tapering to fine points. The haustorium lies in the midst of a rich nutritive tissue in the integument near the micropylar region and remains active for a long period. The chalazal haustorium (Figs. 31 and 32) becomes more or less bulbous and appears like a pad pressed against the rich nutritive cells found at the chalazal region of the seed. Its activity seems to come to an end earlier than that of the micropylar haustorium, for, in an almost ripe seed it appears to be lodged in a compactly collapsed, and doubtless, an erstwhile rich nutritive tissue.

Between these two haustoria the entire portion of the seed is occupied by the mass of endosperm tissue, lying in which is a large dicotyledonous embryo (Fig. 28). The endosperm cells have conspicuous nuclei and show granular contents. The inner walls of the epidermal cells in the mature seed are thickened and show radial spine-like outgrowths, while the outer wall remains thin (Fig. 28).

Embryo

The zygote elongates rapidly and becomes tubular. The first division of the zygote takes place by a transverse wall (Fig. 13). By one more transverse division a small filamentous proembryo of three cells is formed (Fig. 14).



Sphenoclea zeylanica Gaertn. (FIGS. 21 to 33). Fig. 21. First division of the primary endosperm nucleus. × 1800. Figs. 22-27. Stages in the development of the endosperm and the separation of the micropylar and chalazal haustoria. × 1800 each. Fig. 28. Longitudinal section of a mature seed showing the remnants of the endosperm filled with granular contents, the micropylar and chalazal haustoria and the mature dicotyledonous embryo. × 200. Fig. 29. The four-celled micropylar haustorium at a late stage in the seed. × 900. Fig. 30. The same

in transverse section to show the four uninucleate cells of the micropylar haustorium. × 900. Fig. 31. Two of the cells of the chalazal haustorium in a late stage of the seed. × 1800. Fig. 32. The four uninucleate cells of the chalazal haustorium as seen in taansverse section × 1260. Fig. 33. Diagrammatic scheme showing endosperm development. (Original magnifications are indicated here, but the figures have been reduced to two-thirds in reproduction.)

The terminal cell of the proembryo is the embryonal cell, which, after the first vertical (Fig. 15) and the second and third transverse and vertical walls (Figs. 16 and 17) forms the octant stage. With further growth the embryo becomes large and spherical in which the three histogens, namely, the dermatogen, periblem and plerome become differentiated (Figs. 18, 19 and 20). At about the octant stage two intersecting oblique walls are laid in the second cell of the proembryo (Figs. 17, 18 and 19) to form a group of three cells which constitutes the hypophysis. The cells divide further and complete the periblem and the dermatogen at the base of the embryo (Fig. 20). The suspensor is usually made up of two cells. The mature embryo is straight and dicotyledonous, the stem tip arising in the deep notch between the two cotyledons (Fig. 28).

Summary

The wall of the anther shows three layers below the epidermis. The endothecium is fibrillar and the tapetal cells are binucleate. The pollen grain at the time of shedding is trinucleate.

The ovary is semi-inferior and has numerous anatropous ovules on a massive, axile, entire placenta. The ovules have a single thick integument, the innermost layer of which foms an integumentary tapetum. The embryo-sac is formed according to the normal type. The synergids show pointed apices, with the nuclei situated in the centre. The antipodals are organised as definite cells. The two polar nuclei unite before fertilization in the upper one-third region of the embryo-sac to form the secondary nucleus.

Endosperm is *ab initio* cellular and follows the *Scutellaria*-type of Schnarf. The course of development has been described in detail in the paper. Both the micropylar and chalazal haustoria are prominent structures and each of them consists of four large uninucleate cells containing rich cytoplasm.

The course of development of the embryo has been followed in detail and described in the paper. The formation of the hypophysis takes place by two interesecting oblique walls at the base of the embryo.

In conclusion, we wish to thank Dr. L. N. Rao, Professor of Botany, Central College, Bangalore, for his kind encouragement during the course of

this work. We also wish to thank Mr. S. N. Chandrasekhara Iyer, Systematic Botanist, Coimbatore, for kindly determining the species selected for this study.

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A PRELIMINARY SURVEY OF CHROMOSOME NUMBERS IN SCITAMINEÆ OF BENTHAM AND HOOKER

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I. Introduction

The order Scitamineæ, according to Bentham and Hooker (1888), comprises 4 tribes—*Zingibereæ*, *Maranteæ*, *Canneæ* and *Museæ*. In '*Das Pflanzenreich*' of Engler (1902), all these have been raised to the rank of families. Rendle (1930) has also adopted the same scheme. Hutchinson (1934), however, recognizes 6 families under the order Zingiberales, namely, *Musaceæ*, *Strelitziaceæ*, *Lowiaceæ*, *Zingiberaceæ*, *Cannaceæ* and *Marantaceæ*. Whatever may be the treatment accorded to these (*Musaceæ*, *Zingiberaceæ*, etc.), the naturalness of the entire group is accepted by all. The present investigation is a sort of reconnaissance in this direction, to see how far a study of the chromosome numbers of these plants supports relationships recognized on morphological grounds.

It may however be stated, at the very outset, that owing to difficulties in procuring the materials, this karyological survey had to be confined only

to a limited number of species. Hence the conclusions drawn here are necessarily of a tentative nature only.

Of the 4 tribes of the order Scitamineæ, *Museæ* appears to have received most attention. Tischler (1935-36) has given the chromosome numbers for about 24 species of *Musa* out of a total of about 45 (Hutchinson, 1934); for 7 species of *Heliconia*, 2 of *Strelitzia* and for one of *Ravenala*, viz., *madagascariensis*. On the other hand, nothing is known of *Phenakospermum* (*Strelitziaceæ*) and *Orchidantha*—'the potential orchids', placed by Hutchinson (1934) in the family Lowiaceæ.

The *Zingiberæ* although the largest of the 4 tribes is less known cytologically. Tischler (1935, 1938) gives the chromosome numbers for only 7 genera and 11 species. An attempt to provide further data in this direction was made by Raghavan and Venkatasubban (1943) who studied 24 additional species. Considering the total number of genera and species, however, which according to Willis (1931) are 45 and 800 respectively, the data obtained, so far, are still quite inadequate.

Out of a total of about 40 species of the monogeneric tribe *Canneæ* (Rendle, 1930), chromosome numbers for only 7 species have been recorded.

Of the *Maranteæ*, we know almost nothing. As far as I am aware, the chromosome numbers for only two unidentified species of *Maranta* (Kihara, Yamamoto and Hosono, 1931) have so far been recorded.

The present study includes some representatives of all the tribes. In *Museæ*, *Musa ensete* and 4 species of *Heliconia* have been studied. Under *Zingibereæ*, 5 species of *Costus*, 2 of *Kæmpfera*, 2 of *Curcuma* and 1 each of *Alpinia*, *Hedychium* and *Amomum* have been examined. Under *Canneæ* chromosome numbers have been determined for *Canna edulis*. Regarding *Maranteæ*, 4 genera and 20 species have been studied.

II. Materials and Methods

Materials for the present study were obtained in the form of rhizomes or young plants, chiefly, from the Peradeniya Botanic Gardens, Ceylon. As the identifications could not be checked by me, the names under which the rhizomes and young plants were received from their respective sources have been retained as such. For sections, young root-tips were selected, washed in water with the help of a fine camel-hair brush and fixed in Navaschin's fluid for a period of 24 hours. Invariably a prefixation of about 45 seconds was given in Carnoy's fluid (6 parts of absolute alcohol, 3 parts of chloroform and 1 part of glacial acetic acid). Dehydration, infiltration and embedding were done in the customary manner and sections were cut at a

thickness of 14 to 18 microns. Both Feulgen's and Newton's Iodine Gentian Violet method were tried but the latter was found to be superior for chromosome counting.

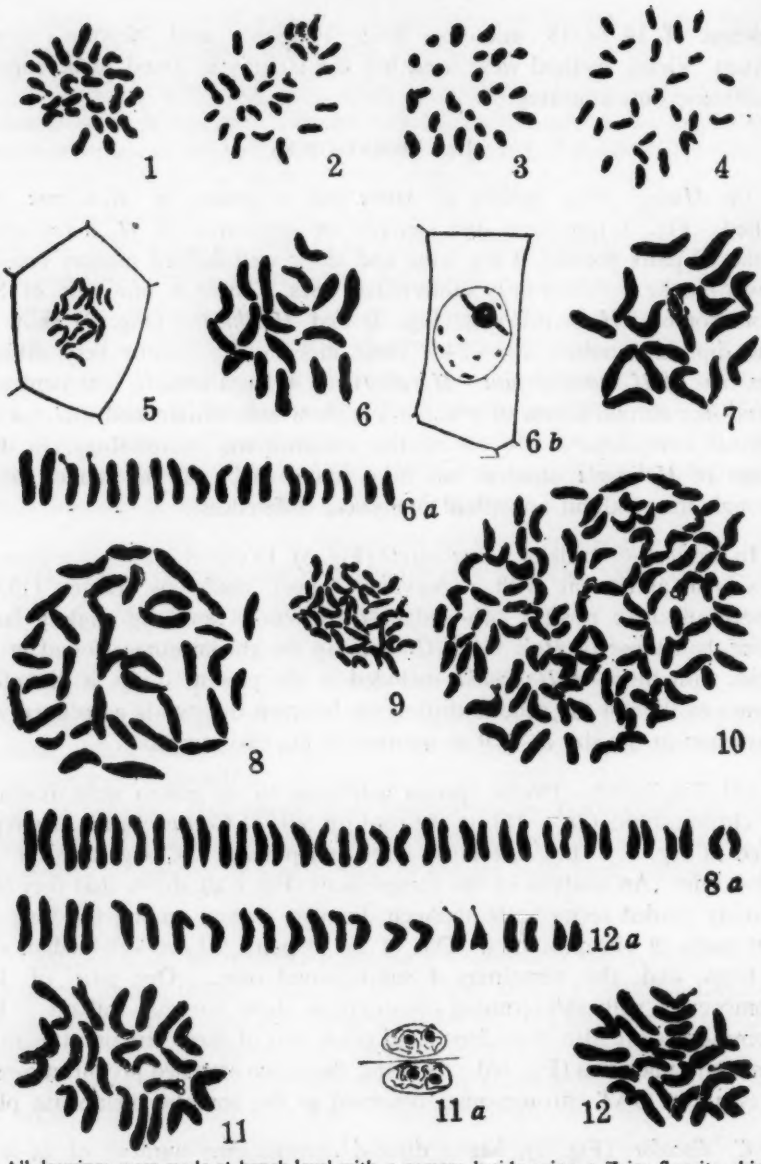
III. Observations

(*a*) *Museæ.*—One species of *Musa* and 4 species of *Heliconia* were studied. Fig. 1 represents the somatic chromosomes of *H. aureo-striata*. Of the 12 pairs present, 4 are large and show well-defined median constrictions while the rest show only subterminal ones. There is one pair of SAT chromosomes. *H. brasiliensis* (Fig. 2) and *H. illustris* (Fig. 3) show the same diploid number, $2n = 24$. Their morphology is not very different from that of *H. aureo-striata*. *H. rubra* (Fig. 4) has a somatic complement of 26 rod-like chromosomes of which some show sub-median and the rest subterminal constrictions. In short, the chromosome morphology, in the 4 species of *Heliconia*, studied, has been found to be of the same pattern although they exhibit aneuploid numerical differences.

In the root-tip cells of *Musa ensete* (Fig. 5), 18 chromosomes are counted. [This is in agreement with a previous report made by Larter (1935).] Almost all these possess sub-median constrictions and are slightly larger in size than those of *Heliconia*. Comparing the chromosomes found in this species with those of *Heliconia*, included in the present study, it, therefore, appears as though the generic distinction between the two is associated with a variation in the size as well as number of the chromosomes.

(*b*) *Zingibereæ.*—Twelve species belonging to six genera were available. The chromosomes ($2n = 18$), in the root-tip cells of *Costus elegans*, are represented in Fig. 6. These are fairly large and perhaps the largest so far seen in the order. An analysis of the complement (Fig. 6 *a*) shows that they form a closely graded series without much disparity in size among the long and short pairs of chromosomes. Out of the 9 pairs 5 have sub-median constrictions and the remaining 4 sub-terminal ones. One pair of long chromosomes with sub-terminal constrictions show terminal trabants. Prochromosomes are also in evidence and often two of these remain attached to the solitary nucleolus (Fig. 6*b*). Perhaps, these two attached prochromosomes represent the SAT chromosomes observed in the somatic metaphase plate.

C. discolor, (Fig. 7), has a diploid chromosome number of $2n = 18$. There is a close agreement with *C. elegans*, although the former did not show the presence of a pair of SAT chromosomes. The complement reveals somatic pairing to a certain extent.



All drawings were made at bench level with a camera lucida using a Zeiss fluorite objective 100 in conjunction with different eyepieces—K. 30, K. 20, K. 17 and K. 10 and reduced to 2/3 in reproduction. All the figures represent stages of Root-tip mitosis in the different plants of *Scitamineæ*.

FIGS. 1–12.—Figs. 1, 2, 3 and 4. Somatic metaphase plates of *Heliconia aureo-striata* ($2n = 24$), *H. brasiliensis* ($2n = 24$), *H. illustris* ($2n = 24$) and *H. rubra* ($2n = 26$) × 5200.

Fig. 5. *Musa ensete* ($2n = 18$) × 2500. Fig. 6. *Costus elegans* ($2n = 118$) × 5200; Fig. 6 *a*. Idiogram of the same. Fig. 6 *b*. Early prophase of *C. elegans* showing the attachment of two nucleolar chromosomes, × 1700. Figs. 7 and 8. *C. bicolor* ($2n = 18$) and *C. afer* ($2n = 36$), × 5200. Fig. 8*a*. Idiogram of *C. afer*. Figs. 9 and 10. Somatic chromosomes of *C. pictus* ($2n = 36$), × 2500 and *C. musaicus* ($2n = 102, 108$), × 5200. Fig. 11. *Kæmpfera speciosa* ($2n = 22$); × 5200; Fig. 11 *a*. Early somatic telophase of the same showing 2 nucleoli in each nucleus, × 1700. Figs. 12 and 12 *a*. Somatic chromosomes and idiogram of *K. atrovirens* ($2n = 22$), × 5200; SAT chromosomes are seen in the complement.

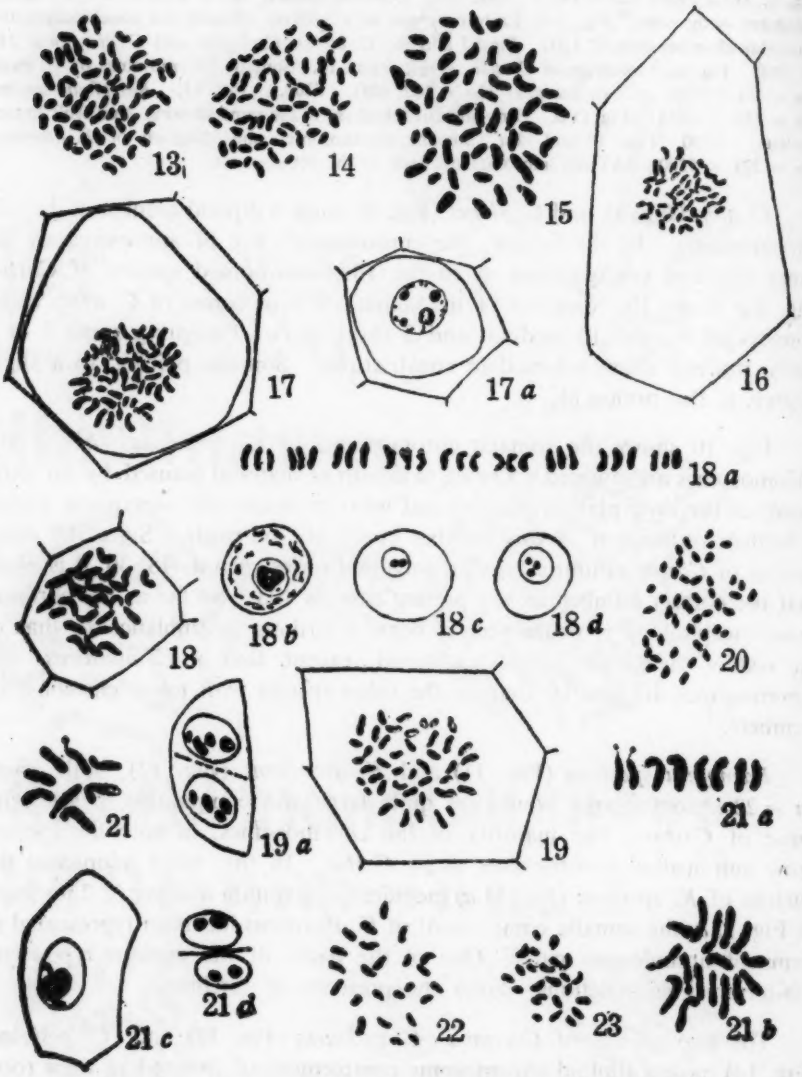
C. afer, (Fig. 8), and *C. pictus*, (Fig. 9), show a diploid number of $2n = 36$ chromosomes. In the former, the chromosomes are of approximately the same size and configuration as in the 18-chromosomed species of *Costus*. Fig. 8 *a* shows the idiogram of the somatic chromosomes of *C. afer*, which consists of 5 long, 10 medium and 3 short pairs. Excepting some 3 or 4 pairs, the rest show sub-median constrictions. Somatic pairing, to a slight degree, is also noticeable.

Fig. 10 shows the somatic chromosomes of *C. musaicus*. About 102 chromosomes are counted. Owing to dearth of material (caused by an early death of the only plant available) and want of favourable metaphase plates, a further verification of this number could not be made. Since the other species of *Costus* exhibit a regular polyploid series such as 18, 36, it is likely that the correct number, in the present case, is 108. So far as the chromosome morphology is concerned, it bears a striking resemblance to that of the rest of the *Costus* species examined, except, that in *C. musaicus*, the chromosomes are smaller than in the other species with lower chromosome numbers.

Kæmpfera speciosa (Fig. 11) and *K. atrovirens* (Fig. 12) both show $2n = 22$ chromosomes, which are quite large and comparable in size with those of *Costus*. The majority of the chromosomes, in both the species, show sub-median constrictions as in *Costus*. In the early telophase, the nucleus of *K. speciosa* (Fig. 11 *a*) includes a maximum number of 2 nucleoli. In Fig. 12 *a*, the somatic complement of *K. atrovirens* has been represented in terms of homologous pairs. One of the pairs, of the medium-type, with sub-terminal constrictions, shows the presence of satellites.

The two species of *Curcuma*—*C. zedoaria* (Fig. 13) and *C. petiolata* (Fig. 14) have a diploid chromosome complement of $2n = 64$ in their root-tip cells. In both cases, the chromosomes are quite small and rod-like offering a good comparison with those of the tribe *Maranteæ*.

Hedychium angustifolium ($2n = 52$). The somatic chromosomes of this species, (Fig. 16), are rod-shaped and slightly larger than those of *Curcuma*.



FIGS. 13–23. Figs. 13 and 14. Somatic metaphase chromosomes of *Curcuma zedoaria* and *C. petiolata* ($2n = 64$) × 5200. Fig. 15. *Hedychium angustifolium* ($2n = 52$) × 5200. Figs. 16 and 17. *Alpinia sanderiana* ($2n = 48$) and *Amomum magnificum* ($2n = 48$) × 2500. Fig. 17 *a*. Shows a telophase nucleus of the latter species with 4 nucleoli some big and some small with attached prochromosomes × 1700. Fig. 18. *Canna edulis* ($2n = 3 \times = 27$) × 2500. Fig. 18 *a*. Idiogram of the same showing 9 sets of 3 each. Fig. 18 *b*. Shows prochromosomes, crystalline inclusion and two chrocentres of *Canna edulis*. Figs. 18 *c*, *d*. Show respec-

tively, 2 and 3 crystalline inclusions of the nucleolus of the same species, × 1700. Fig. 19. *Stromanthe sanguinea* (2*n* = 44), × 3400. Fig. 19 *a*. Telophase of the same showing 4 nucleoli, × 1700. Fig. 20. *Phrynium arundinacea* (2*n* = 46), × 3400. Fig. 21. *Maranta nitida-picta* (2*n* = 8); 2 SAT chromosomes are seen, × 5400. Fig. 21 *a*. Idiogram of the same; Fig. 21 *b*. Tetraploid cell from the periblem region of the same species (4 × 16), × 5200. Fig. 21 *c*. Shows the two nucleolar chromosomes of the same species, × 1700. Fig. 21*d*. Showing the two telophase nuclei, × 1700. Figs. 22 and 23. *Maranta bicolor* (2*n* = 24); *M. Leitzii* (2*n* 24), × 3400.

Alpinia sanderiana.—This species shows 48 small rod-shaped chromosomes (Fig. 15), which are quite similar to those of *Curcuma*.

Amomum magnificum (Fig. 17).—48 chromosomes are counted. These are slightly larger in size compared to those of *Alpinia sanderiana*. The complement includes a pair of SAT chromosomes. The telophase nuclei (Fig. 17 *a*) show a maximum of 4 nucleoli, out of which one is bigger than the rest. A prochromosome is found attached to each one of these.

(*c*) *Cannæ*.—*Canna edulis*.—In the root-tip cells of the plant examined, (Fig. 18), 27 chromosomes are counted. These have been analysed into their homologues and represented in Fig. 18 *a*. Though no critical analysis could be made, the 27 chromosomes appear to fall into 9 sets, of 3 each, and the sets themselves form a graded series. The form in question, therefore, appears to be a triploid. Belling (1927) gave 9 as the haploid number for *Canna edulis*.

The nucleolus shows some interesting inclusions. In most cases, only one prominent nucleolus is seen (Fig. 18 *b*). Inside this are observed some bodies with a crystalline appearance which remained unstained with Gentian Violet. Sometimes, only one such body is seen (Fig. 18 *b*), sometimes two and in some cases even three (Figs. 18 *c, d*). The increase in their number is associated with a decrease in their size. In some preparations, these appeared to be in the act of fusing (Fig. 18 *c*). Besides such bodies, the nucleolus also exhibited other small rounded bodies which did stain with Gentian Violet. In Fig. 18 *b* two such bodies are seen one on either side of the crystalline inclusion. Very likely these are in the nature of chromocenters. Prochromosomes are also met with.

(*d*) *Marantæ*.—Species belonging to 4 genera were studied:—*Stromanthe sanguinea, Phrynium arundinacea* and a number of species of *Maranta* and *Calathea*.

Stromanthe sanguinea (2*n* = 44).—The somatic chromosomes, (Fig. 19), are quite small and comparable in their size to those of *Amomum* or *Curcuma* species. The telophase nuclei (Fig. 19 *a*) show 4 nucleoli; but in the resting stage only one could be observed, presumably, due to a fusion of the former.

Phrynium arundinacea.—Fig. 20 shows the 46 minute chromosomes constituting the somatic complement of this species.

Maranta nitida-picta.—Only 8 chromosomes, (Fig. 21), are observed in the root-tip cells of this species. These are found to be three times as large as those observed in other species of the same genus. This number ($2n = 8$) is the lowest, so far recorded in the family Scitamineæ. From the idiogram (Fig. 21 *a*), it can be seen that 3 pairs show sub-median constrictions, while the 4th pair has sub-terminal ones. This sub-terminal pair bears satellites at its proximal end. The 4 pairs do not vary much in their size. Tetraploid cells with $2n = 4X = 16$ chromosomes are also frequently encountered in the region of the periblem (Fig. 21 *b*). In the early prophase a pair of nucleolar chromosomes, (Fig. 21*c*), is observed and the early telophase, (Fig. 21 *d*), regularly showed the presence of 2 nucleoli.

In the following species of *Maranta* 24 chromosomes are counted during the metaphase of root-tip mitoses:—*Maranta bicolor* (Fig. 22); *M. Lietzii* (Fig. 23); *M. asymetrica* (Fig. 24); *M. zebrina* (Fig. 25) and *M. tigrina* (Fig. 26). The chromosomes, in all these, are very small like those found in *Curcuma* or *Alpinia*. They agree not only in a particular complement but also with regard to the complements of the different species examined.

The following species of *Maranta* show a diploid chromosome number of $2n = 26$:—*Maranta nitida* (Fig. 27), *M. roseo-picta* (Fig. 28), *M. makoyana* (Fig. 29), *M. Massangeana* (Fig. 30), and *M.* sp. (unidentified) (Fig. 31). The chromosomes in these species are quite small and agree with those of the 24-chromosomed forms. It is only in the last-named and unidentified species of *Maranta* that they are somewhat larger than the rest. In this there is one pair which is quite conspicuous, not only on account of its satellited nature but also on account of its size.

The several species of *Maranta* also show the presence of prochromosomes; *M. zebrina* (Fig. 25 *a*) and *M. tigrina* (Fig. 26 *a*). In both cases it was further observed that two of the prochromosomes regularly attached themselves to the nucleolus.

Striking resemblances were noted in the appearance of chromosomes of some of the species of *Maranta*, to those of the tribe *Canneæ*, *Zingibereæ* and *Museæ*. The forms showing a close resemblance in chromosome morphology are:—*Maranta Massangeana*, *Musa ensete* (compare Figs. 5 and 30), *M. tigrina*, *Alpinia sanderiana* and *Curcuma petiolata* (Figs. 26, 14 and 16); *Maranta asymetrica*, *M. zebrina* and *M. nitida* (Figs. 24, 25 and 27); *M. roseo-picta*, *Hedychium angustifolium* (Figs. 15 and 28); *Stromanthe sanguinea*, *Amomum magnificum* and *Curcuma zedoaria* (Figs. 19, 17 and 13).

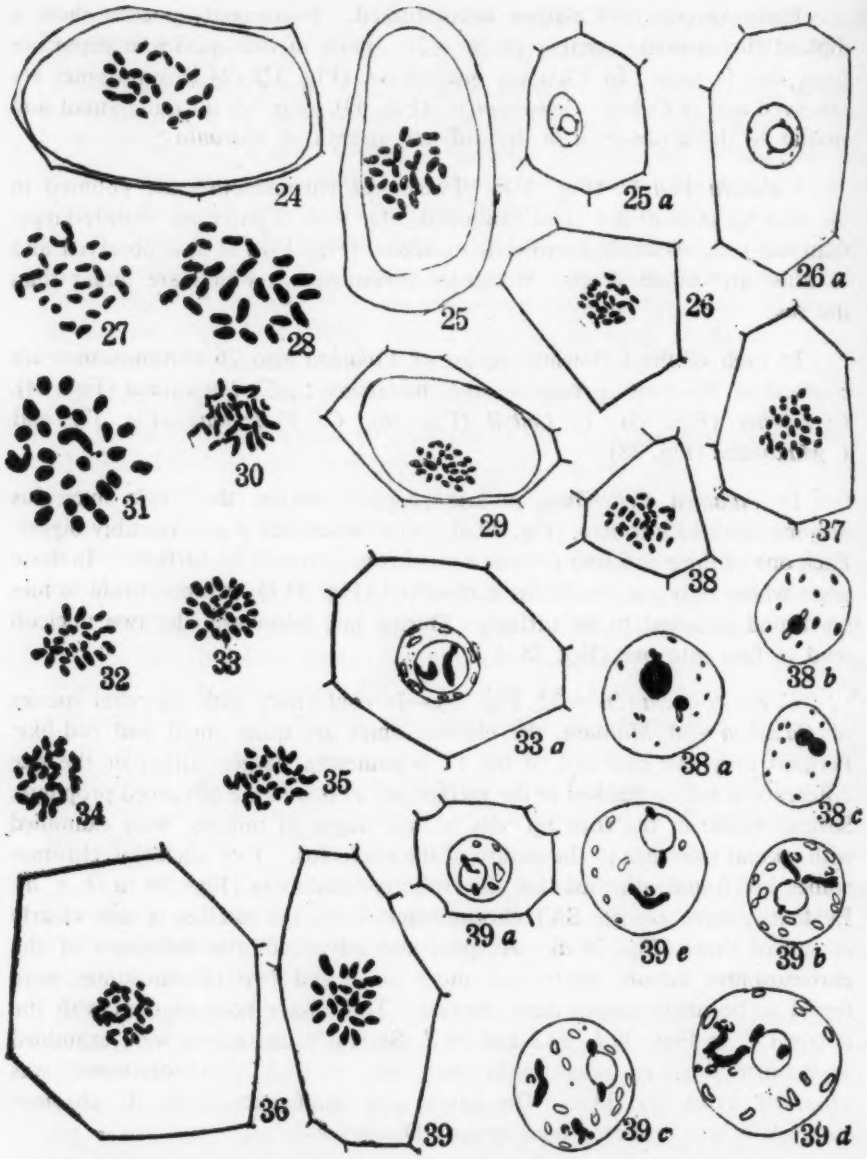
Eight species of *Calathea* were studied. Their root-tip cells show a diploid chromosome number of $2n = 26$. Only in two species, a departure from this is seen. In *Calathea grandiflora*, (Fig. 32), 24 chromosomes are observed and in *Calathea medeo-picta*, (Fig. 39), only 22, all quite small and similar to those observed in the different species of *Maranta*.

Calathea Veitchii (Fig. 33): Twenty-six chromosomes are counted in the root-tip cells of the plant examined. Of these 2 pairs are slightly larger than the rest. A single prominent nucleolus (Fig. 33 *a*) is also observed and to this are attached two nucleolar chromosomes which are larger than the rest.

In each of the following species of *Calathea* also 26 chromosomes are counted in the root-tip cells in the metaphase:—*C. Lindeniana* (Fig. 34), *C. zebrina* (Fig. 35), *C. Leitzii* (Fig. 36), *C. Veitchiana* (Fig. 37) and *C. Makoyana* (Fig. 38).

In *Calathea Veitchiana*, in late prophase stages, the single nucleolus becomes divided into two, (Fig. 38 *a*), out of which one is considerably bigger. Each one of these is found to bear a prochromosome on its surface. In those cases where only one nucleolus is observed (Fig. 38 *b*), two prochromosomes are found attached to its surface. During late telophase, the two nucleoli tend to fuse into one (Fig. 38 *c*).

C. medeo-picta ($2n = 22$, Fig. 39).—In conformity with the other species of *Calathea* and *Maranta*, the chromosomes are quite small and rod-like. Perhaps only one pair out of the 11 is somewhat larger, although the size difference is not so marked in the metaphase as it is in the advanced prophase. Several nuclei in the root tip cells, at all stages of mitosis, were examined with special reference to the nature of the nucleolus. Two nucleolar chromosomes are found attached to the solitary nucleolus (Figs. 39, *a*, *b*, *c*, *d*). Evidently, these are the SAT chromosomes since the satellite is seen clearly in one of these (Fig. 39 *a*). As prophase advanced, the definition of the chromosomes became more and more clear and two chromosomes were found to be much longer than the rest. These have been marked with the letters CC′ in Figs. 39 *b*, 39 *c* and 39 *d*. Several preparations were examined to confirm this and occasionally only one such 'C' chromosome was observed, as in Fig. 39 *e*. The nature and significance of the 'C' chromosomes will be considered later under 'Discussion'.



FIGS. 24–39. Figs. 24, 25 and 26. *Maranta asymetrica* ($2n = 24$), *M. zebrina* ($2n = 24$), × 3400; and *M. tigrinia* ($2n = 24$), × 2500. Figs. 25 *a* and 26 *a* show prochromosomes and 2 nucleolar chromosomes in *M. zebrina* and *M. tigrina*, × 1700. Figs. 27, 28, 29, 30, 31, 32 and 33. Somatic chromosomes of *M. nitida* ($2n = 26$), *M. roseo-picta* ($2n = 26$), × 3400;

M. Makoyana (2*n* = 26), *M. Massangeana* (2*n* = 26), × 2500; *Maranta* sp., (2*n* = 26), × 5200; *Calathea grandiflora* (2*n* = 24), *C. Veitchii* (2*n* = 26) × 2500. Fig. 33 *a*. *C. Veitchii* showing 2 nucleolar chromosomes, × 2500. Figs. 34, 35, 36, 37 and 38. *C. Lindeniana* (2*n* = 26), *C. zebrina* (2*n* = 26), *C. Leitzii* (2*n* = 26), *C. Veitchiana* (2*n* = 26) and *C. Makoyana* (2*n* = 26), × 2500. Fig. 38*a*. *C. veitchiana* showing two nucleoli, one big and the other small, each with an attached prochromosome, × 2500. Fig. 38 *b*. The same species showing prochromosomes two of which are found attached to the single nucleolus, × 2500; Fig. 38 *c* shows the fusion of two nucleoli and prochromosomes in the same species, × 2500. Fig. 39. *C. medeo-picta* (2*n* = 22), × 2500. Fig. 39 *a*. The same species showing 2 nucleolar chromosomes of which one shows the presence of a satellite, × 1700. Fig. 39 *b*. Prophase in the root-tip cells of the same material showing CC′ chromosomes, × 2500. Figs. 39 *c* and *d*. Prophase stages in the root-tip mitosis of the same species showing the CC′ chromosomes and 2 nucleolar chromosomes. Fig. 39 *e*. The same species showing only one 'C' chromosome, × 2500.

The following will sum up the chromosome numbers determined in the present study:—

1. MUSEÆ

Name of the Plant	Source	2*n*
Musa enset	Tenmalai forests, Travancore.	18
Heliconia illustris, Hort.	Lal-Bagh, Bangalore	24
H. aureo-striata, Hort.	,, ,,	24
H. rubra Sesse & Moc.	,, ,,	26
H. brasiliensis, Hook.	Peradeniya Gardens, Ceylon	24

2. ZINGIBEREÆ

Costus elegans Hort.	Peradeniya Gardens, Ceylon	18
C. bicolor. F. Baraun	Soundarya Nursery, Madras	18
C. afer	Peradenyia Gardens, Ceylon	36
C.-pictus	,, ,,	36
C. musaicus Hort.	,, ,,	102 ? 108
Kæmpfera speciosa Baker	,, ,,	22
K. atrovirens N. E. Br.	,, ,,	22
Curcuma zedoaria Roscoe	Sibpur Gardens, Calcutta	64
C. petiolata Roxb.	,, ,,	64
Hedychium angustifolium Roxb.	Peradeniya Gardens, Ceylon	52
Alpinia sanderiana Hort.	,, ,,	48
Amomum magnificum Benth. & Hook.	,, ,,	48

3. CANNEÆ

Canna edulis Ker-Gawl	Peradeniya Gardens, Ceylon	27

4. MARANTEÆ

Stromanthe sanguinea Sond.	Peradeniya Gardens, Ceylon	44
Phrynium arundanacea	Alipur Agri-Horticultural Gardens, Calcutta	46
Maranta nitida-picta	Lal-Bagh, Bangalore	8
M. roseo-picta Hort.	,, ,,	26
M. nitida	,, ,,	26

Name of the Plant	Source	2 *n*
M. makoyana	Peradeniya Gardens, Ceylon	26
M. Massangeana Hort.	,, ,,	26
M. tigrina	,, ,,	24
M. zebrina	,, ,,	24
M. bicolor Ker-Gawl	Lal-Bagh, Bangalore	24
M. asymetrica Hort.	,, ,,	24
M. Lietzei Hort.	Peradeniya Gardens, Ceylon	24
Calathea grandiflora Donn.	,, ,,	24
C. Leitzii E. Morr.	,, ,,	26
C. Makoyana E. Morr.	,, ,,	26
C. Veitchii	,, ,,	26
C. Veitchiana, Veitch	,, ,,	26
C. Lindeniana Wallis.	,, ,,	26
C. zebrina Lindl.	,, ,,	26
C. medeo-picta	,, ,,	22

TABLE I

Chromosomal frequency in Museæ

Names of Genera	*n*=4	5	6	7	8	9	10	11	12	13	16
Musa ..						2	4	14	1		2
Heliconia ..					1	1	1	2	8	1	
Strelitzia ..				1				1			
Ravenalia ..				1							
Balcana ..				1							
Total *n* = ..				3	1	3	5	17	9	1	2

TABLE II

Chromosomal frequency in Canneæ

	n=9	27/2
Canna ..	7	3

TABLE III

Chromosomal frequency in Marantea

Genera	*n*=4	11	12	13	16	22	23
Maranta ..	1		6	4	1		
Calathea ..		1	1	6			
Stromanthe ..						1	
Phrynium ..							1
Total ..	1	1	7	10	1	1	1

TABLE IV

Chromosome Number distribution in Zingibereæ

Genera	$n=8$	9	11	12	17	18	21	24	26	27	31	32	33	64
Costus ..	1	5				3								1
Zingiber ..			3	1										
Kæmpfera ..			2	1		1				2				
Hedychium ..					1	1			2	2			2	
Curcuma ..							1				1	3		
Alpinia ..								6						
Elettaria ..								1						
Phæomeria ..								1						
Globba ..								1						
Amomum ..								1						
Total ..	1	5	5	2	1	5	1	10	2	4	1	3	2	1

IV. DISCUSSION

(*a*) *Chromosomal Distribution in Scitamineæ.*—The frequency of chromosome numbers in the different tribes is indicated in Tables I, II, III and IV. More than 50% of the total number of species of the genus *Musa* (Table I) have 11 as their haploid number; a little over 20% have 10 and a few have 9 and 16. In the genus *Heliconia*, out of a total of 14 species investigated, as many as 8 show the chromosome number $n = 12$ and each one of the rest has a different number ranging from $n = 8$ to $n = 13$. In the rest of the genera, like *Strelitzia*, *Ravenalia* and *Babcana*, $n = 7$ appears to be the prevailing number. Therefore the numbers $n = 7$, 10, 11 and 12 appear to be the dominant ones.

The members of the tribe *Zingibereæ* show a wider range from $n = 8$ to $n = 54$. In some cases, each genus seems to have adopted a particular number. Thus, all the species of *Costus*, with a single exception, have a haploid chromosome set of 9 or its multiples. Similarly the number 11 characterizes most of the species of *Zingiber*. There are also a number of genera which exhibit the same haploid number, $n = 24$ as in *Alpinia*, *Elettaria*, *Phæomeria*, *Globba* and *Amomum*. On the other hand, species of the same genus show different numbers. Thus in *Kæmpfera*, the numbers $n = 11$, 12, 18 and 27 have been recorded. Similarly, *Curcuma* has $n = 21$ or 31 or 32 and in *Hedychium*, we have, $n = 17$, 18, 26, 27 and 33.

In the tribe *Canneæ* (Table III), the prevailing number is $n = 9$ and $2n = 3X = 27$.

The members of the second biggest tribe of *Scitamineæ—Maranteæ* also show a wide variation in their chromosome numbers, although this is not of the same magnitude as in *Zingibereæ*. The chromosome numbers vary from $n = 4$ to $n = 23$ in the different genera. In species of the genus *Maranta*, the occurrence of $n = 12$ and $n = 13$ has been found to be about equally common, while in the majority of the species of *Calathea*, $n = 13$ was found to be predominant. It may be recalled in this connection, that these two genera belong, respectively, to the tribes *Maranteæ* and *Phrynieæ* (Hutchinson, 1934). These tribes also include other genera like *Stromanthe* ($n = 22$) and *Phrynium* ($n = 23$). The genus *Maranta*, has also an added interest, namely, that one of the species—*M. nitida-picta* shows $n = 4$ chromosomes, which is not only the lowest chromosome number found in the *Maranteæ* but in the whole order *Scitamineæ*.

(*b*) *Polyploidy.*—The importance of polyploidy as a factor in evolution needs no recounting. The works of Winge (1917, 1924), Clausen (1927), Muntzing (1936) and Anderson and Sax (1936) have provided ample evidence of the significant part played by polyploidy in the evolution of new species. So far as Scitamineæ are concerned, typical polyploid series, such as are found in *Salix*, *Solanum*, *Senecio*, *Chrysanthemum*, *Triticum*, etc., are almost wanting. Such typical series, so far as the present data go, is found only in the genus *Costus*, where chromosome numbers like 18, 36 and 108 have been encountered. This is also true of the different species of *Canna*, where also such numbers as 18 and 27 are met with. However, one can still infer the polyploid nature, of at least the higher chromosomed-species, from indirect evidence such as the number of nucleoli during early telophase or the number of prochromosomes or nucleolar chromosomes attached to the nucleolus or nucleoli, as the case may be, in the earlier stages of mitosis. Thus, for example, one can interpret *Amomum magnificum* ($2n = 48$) as a tetraploid, for, here in the early telophase, 4 nucleoli and 4 prochromosomes attached to these have been observed. Similar indirect evidence is present regarding the tetraploid nature of *Stromathe sanguinea*. A detailed study of some of the members of the tribe *Zingibereæ* has led Raghavan and Venkatasubban (1943) to conclude that polyploidy is prevalent in species belonging to several genera such as *Alpinia*, *Phæomeria*, *Elettaria* and *Globba*. These are considered to be tetraploids derived from ancestral forms with 12 somatic chromosomes. Similarly, the different species of *Kæmpfera* are supposed to represent polyploid forms derived from the base number $n = 6$. The different species of *Hedychium* are considered to have been derived from forms with 13 chromosomes; *Curcuma aromatica* ($n = 21$) is likely to be a triploid form of *C. longa* ($n = 31$) while the 42-chromosomed *C. amada* and

C. aromatica may be amphidiploids which have originated from a cross between some 12-chromosomed and 9-chromosomed ancestors. Further, if it is conceded that the basic numbers for the family are 4, 5 and 6, then, it follows that most of the members included under this order have to be interpreted as polyploids. There is also another reason, why there should be more polyploids in this family than appears to be the case to a casual observer. *Scitamineæ* is essentially a tropical family and extremes of heat or cold could easily induce polyploidy, as has been shown by Hagerup (1932) and Tischler (1935). Besides exhibiting polyploidy, the members of this family also show aneuploidy.

(*c*) ***Aneuploidy.***—The different species of *Musa* show such aneuploid numbers as $n = 9$, 10, 11, 12, etc., *Heliconia* species also exhibit a similar aneuploid series; $n = 8$, 9, 10, 11, 12 and 13. In the tribe *Zingibereæ*, a few genera like *Kæmpfera* ($n = 11$, 12), *Zingiber* ($n = 11$, 12), *Hedychium* ($n = 17$, 18, 26, 27) and *Curcuma* ($n = 31$, 32) reveal such aneuploid relationship. Aneuploidy is also found in the tribe *Maranteæ*. Species of the genera *Maranta* and *Calathea* exhibit both 24 and 26 chromosomes in their root-tip cells. The occurrence of such aneuploidal relationship among the species, as has been pointed above, is interesting, since according to Manton (1932), the presence of aneuploid numbers within the same genus is rather an uncommon phenomenon. Nevertheless, a similar occurrence has been noted in another family also. The genus *Tecoma*, for instance, provides an unmistakable evidence of a beautiful aneuploid series such as $2n = 34$, 36, 38 and 40 (Venkatasubban, 1944). Although aneuploid relationship has been found within the genus of this family, it is much more frequent among the different genera. To give only a few examples, *Musa* has $n = 11$ while *Heliconia* has $n = 12$. A few species of *Heliconia* also include the number $n = 8$ while other genera like *Strelitzia*, *Ravenalia* and *Babcana* show $n = 7$. Again, in the *Zingibereæ* the genus *Zingiber* has the haploid number 11 while *Kæmpferia* has 12 (no doubt there are species of *Kæmpferia* which show other numbers like 18 and 27). *Hedychium* has $n = 26$ and there are species of *Kæmpfera* exhibiting the number $n = 27$; *Curcuma* species with $n = 32$ and *Hedychium* species showing $n = 34$. In the tribe *Maranteæ*, *Maranta* has $n = 12$ while *Calathea* has $n = 13$. Similarly, *Stromanthe* has $n = 22$ while species of *Phrynium* shows $n = 23$. Thus it appears that aneuploidy is much more frequent among the members of this family than polyploidy.

(*d*) *Compound Chromosomes.*—Compound chromosomes, although common in animal kingdom, are of rare occurrence among plants. Tanaka (1939) has recorded the presence of a compound chromosome (composed of

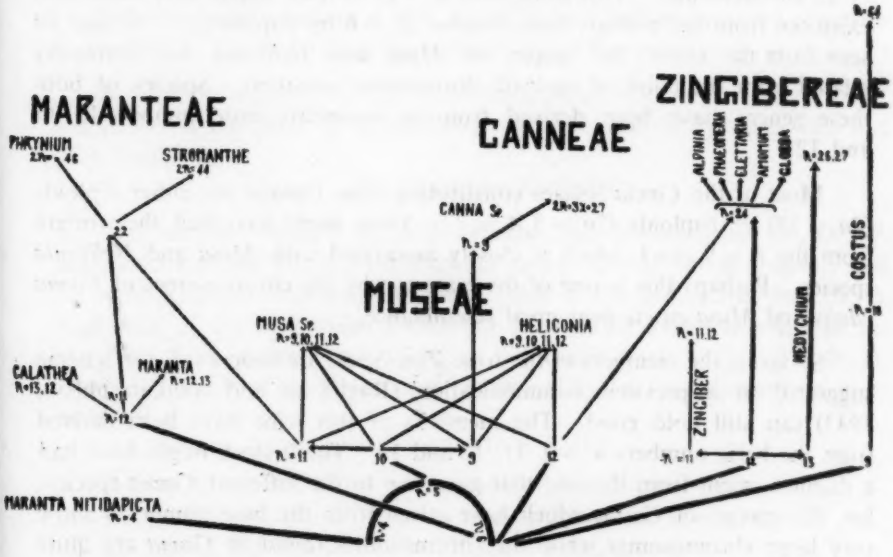
3 chromosomes) in *Scirpus lacastris* var. *pictus*. This makes one suspect that the two big chromosomes found in *Calathea medeo-picta* (Fig. 39, *b*, *c* and *d*) may also be compound structures. While all the other investigated species of *Calathea* show a diploid number of $2n = 24$ or 26, this species alone shows $2n = 22$ chromosomes. Now, if these two big chromosomes are conceded as representing two chromosomes each, this species would be brought into conformity with the other 24-chromosomed species. Since in some cases only one such compound chromosome is met with, it is likely that some of the root-tip cells of this species show $2n = 23$ chromosomes. The two big SAT chromosomes in the unidentified species of *Maranta* (Fig. 31) are also likely to be in the nature of compound chromosomes. Further cytological evidence is, however, necessary to establish the correctness of this view.

(*e*) ***Base Numbers and Phylogeny.***—A detailed study of some members of the tribe *Zingibereæ* led Raghavan and Venkatasubban (1943) to suggest the possibility of $X = 6$ as the primary basic number and $X = 9$, 11, 12 and 13 as secondary balances of the primary basic number. The primary basic number $X = 6$ was then considered as purely hypothetical, in view of the high chromosome numbers met with among the investigated species of the *Zingibereæ*. However, the actual occurrence of $n = 4$ in one of the *Maranta* species, in this investigation (*M. nitida-picta*) gives one the hope of obtaining the basic number $X = 6$ also. This is likely to be realized when a more complete record of the chromosome numbers for this order becomes available.

Most of the members of the tribe *Museæ* and *Canneæ* exhibit such numbers as $n = 9$, 10, 11 and 12 and as these values are somewhat high to represent the base numbers themselves, it is suggested that these have probably arisen from a primary basic number 5. This primary basic number 5, by the simple process of duplication, could have given rise to 10, which is quite likely, since some of the species of *Maranta* actually show tetraploid cells (*M. nitida-picta*). Similarly, by the duplication of the entire set of chromosomes, $X = 12$ could be obtained from the basic number $X = 6$. When once such a duplication is effected, the resultant chromosomes may undergo either a loss of one chromosome (by fusion of two chromosomes) or a gain of one by the process of splitting or duplication. The result of such changes would lead to the establishment of a regular aneuploid series like 9, 10, 11 or 11, 12 and 13. The frequent occurrence of aneuploid series both among the different genera and within the species of the same genus of this order seems to support this assumption. Therefore, it is suggested that $X = 4$, 5 and 6

constitute the primary basic numbers of this order and that secondary balances like X = 9, 10 and 11 or 11, 12 and 13 have been established partly by duplication and in part by fusion or duplication of one of the chromosomes concerned. It can be seen from the appended chart that the chromosome numbers for most of the investigated species can be derived either directly from the basic numbers, 4, 5 and 6 or indirectly from the secondary balances like 9, 10, 11, 12 and 13.

An attempt has been made to assemble the members of the different tribes on the basis of chromosome numbers and size. The four tribes have been kept as such and their interrelationship through chromosome numbers is shown in the appended chart. The members of the tribe *Maranteæ* have



n =6

been derived from 4 sources: Those which show a haploid number of $n = 4$ (*M. nitida-picta*) have been derived directly from the primary base number 4. Those showing $2n = 24, 26$ (*Maranta* sp., and *Calathea* sp.) from the primary base number 6 through a process of duplication of the entire genome. Such forms as *Stromathe* and *Phrynium* with 44 and 46 chromosomes in their root-tip cells have been derived from two sources:—The *Maranta Calathea* stock with $n = 12$ through fusion of two chromosomes might result in a stock with $n = 11$ which might in turn produce $n = 22$. It is also likely that some of the 44-chromosomed members of the *Maranteæ* might have had their

origin in the $n = 11$ stock, which gave rise to some of the species of *Musa*. This alternative is suggested in view of the fact that the chromosomes of *Stromanthe* bear a close resemblance to those found in species of *Musa* and *Heliconia*.

The majority of the species in *Museæ* come under the genera *Musa* and *Heliconia*. In the former $n = 11$ is dominant while in the latter majority of the species exhibit $n = 12$. All the *Musa* species have been derived from the primary basic number $n = 5$ by duplication and further changes leading to the establishment of aneuploid numbers like 9 and 11. Since a few species of this genus also exhibit $n = 12$, these have been derived from the stock which gave rise to the majority of the *Heliconia* species with a haploid set of 12 chromosomes. This stock with $n = 12$ in itself might have come into existence from the primary base number $X = 6$ by duplication. As can be seen from the chart, the species of *Musa* and *Heliconia* are intimately related from the point of view of chromosome numbers. Species of both these genera have been derived from the secondary basic numbers 10, 11 and 12.

Most of the *Canna* species constituting tribe *Canneæ* are either diploids ($2n = 18$) or triploids ($2n = 3 X = 27$). These might have had their origin from the $n = 9$ stock which is closely associated with *Musa* and *Heliconia* species. Perhaps this is one of the reasons why the chromosomes of *Canna edulis* and *Musa ensete* bear great resemblance.

So far as the members of the tribe *Zingibereæ* are concerned, the scheme suggested in a previous communication (Raghavan and Venkatasubban, 1943) can still hold good. The members of this tribe have been derived from the basic numbers $n = 9$, 11, 12 and 13. The 9 stock might have had a distinct origin from the one that gave rise to the different *Canna* species; for, the species of *Costus* which have arisen from the base number 9 show very large chromosomes while the chromosomes found in *Canna* are quite small, if the chromosomes of *Canna edulis* can be regarded as the representative type for the entire genus. The 48-chromosomed *Alpinia* species might have arisen partly from the $n = 12$ *Heliconia* stock and partly from the other stock. This may also apply to the genus *Amomum*; for, in *Heliconia*, *Alpinia* and *Amomum*, the chromosomes are quite small and comparable in their size to one another.

V. Summary

Diploid chromosome numbers for 38 uninvestigated species of *Scitamineæ* have been recorded together with a description of their morphology.

The frequency of chromosome numbers for the different members of this order has been studied.

Indirect evidence has been adduced to show that most of the high chromosome-numbered species of this order are in the nature of polyploids.

Aneuploid numerical relationships has been met with in the chromosome numbers of not only the different genera but also within the species of the same genus.

The two big chromosomes seen regularly during prophase in the root-tip cells of *Calathea medeo-picta* are interpreted as compound structures and the occurrence of 23 chromosomes in some cells of the root-tip has been postulated.

Primary basic numbers $n = 4$, 5 and 6 and secondary basic numbers $n = 9$, 10, 11, 12 and 13 have been suggested for the order.

A phyletic scheme showing interrelationship among the members of this order on the basis of chromosome numbers and chromosome size has been included.

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THE EFFECT OF SOME METALS, VITAMINS, ANÆSTHETICS, AND OTHER SUBSTANCES ON UNSTRIATED MUSCLE

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PART I

Action of Heavy Metals

Many metals are indispensable for life in small concentrations. They are present in the food we eat and in the water we drink and are constituents of protoplasm. Every living organism without exception contains the following elements: carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus, chlorine, potassium, sodium, calcium, magnesium, copper and iron. In addition to these thirteen elements, others are found in certain organisms, sometimes to a large extent. Of these elements we may mention especially silicon, iodine, fluorine, bromine, aluminium, nickel, cobalt, zinc, lead, silver, cadmium, lithium, strontium, and manganese (Fox and Ramage, 1931).

In the present research the action of the following metals on the excitability of unstriated muscle was investigated: arsenic trichloride, uranium nitrate, antimony chloride, bismuth subnitrate, ferric chloride, mercuric chloride, chromium chloride, aluminium chloride, silver nitrate, gold chloride, platinum chloride, copper sulphate, zinc chloride, cadmium chloride, cobalt chloride, nickel chloride, manganese chloride, and lead nitrate. Many of these metals are precipitated by alkaline Ringer solution (pH 8, borate), or by the chlorides but in the concentrations used (I in 10^7–10^4), they were found to be sufficiently soluble to exert definite actions. The action was that of the metal, as the corresponding anions in the concentrations used had no significant effect. The muscle used was mostly that of the frog stomach and was stimulated by alternating current (A.C.), direct current (D.C.), and potassium (0·02 to 0·04 M KCl).

Action of zinc.—Traces of zinc are indispensable for nutrition. Salant and Mitchell (1916) found that the movements of the excised intestines were depressed by very dilute solutions and abolished by higher concentrations. 1 in 10^7 improves the response to A.C., D.C., potassium and acetylcholine in the dog stomach. In the frog stomach it improves the response to A.C.,

D.C., potassium but higher concentrations are depressant. Higher concentrations, 1 in 10^6–10^4 increase the response to potassium and decrease that to electric current, showing a differential effect on the two excitabilities. 1 in 10^6–10^5 increase the response to acetylcholine in dog stomach, but higher concentrations depress it. 1 in 10^4 causes tonic contraction and depresses excitability in general and appears to be toxic.

Action of mercury.—1 in 10^7 improves the response to A.C., D.C., in dog and frog stomachs, but depresses that to potassium; 1 in 10^6 has opposite effects. In dog stomach 1 in 10^7 depresses and 1 in 10^6 improves the response to acetylcholine. 1 in 10^5 is depressant and causes tonic contraction.

Action of cadmium.—The rest of the experiments were performed on the frog stomach. 1 in 10^7–10^6 inhibit tone, decrease the response to potassium, increase that to A.C. and D.C. 1 in 10^5 increases and 1 in 10^4 decreases tone; these concentrations are depressant to A.C. and potassium.

Action of copper.—1 in 10^7 inhibits tone and the response to potassium, but greatly improves the response to A.C. 1 in 10^6 decreases tone and response to A.C., but increases that to potassium. Higher concentrations cause tonic contractions and are depressant to alternating current and potassium.

Action of silver.—1 in 10^7 decreases tone and response to A.C., but increases that to potassium. 1 in 10^6 decreases the response to A.C., potassium and tone; 1 in 10^5 has the opposite action. 1 in 10^4 causes tonic contraction and decreases the response to A.C., and potassium.

Action of gold.—1 in 10^7 improves the response to A.C., and potassium but inhibits tone. 1 in 10^6 decreases the response to A.C., but increases that to potassium; 1 in 10^5–10^4 has opposite action. 1 in 10^3 is depressant to A.C., and potassium, but causes tonic contraction.

Action of aluminium.—1 in 10^7 increases the response to A.C., and potassium, but diminishes tone; 1 in 10^6 decreases all. 1 in 10^5–10^4 causes tonic contraction.

Action of tin.—1 in 10^7–10^5 increases the response to A.C., and decreases that to potassium; 1 in 10^4 has the opposite action and increases tone.

Action of lead.—1 in 10^7 increases the response to A.C., and potassium and decreases tone. 1 in 10^6–10^4 increases the response to A.C., and decreases that to potassium and tone.

Action of arsenic.—1 in 10^7 increases the response to A.C., and potassium but diminishes tone. 1 in 10^6–10^5 decreases all. 1 in 10^4 causes tonic contraction but depresses excitability.

Action of antimony.—1 in 10^7 increases the response to A.C., and potassium but diminishes tone. 1 in 10^6 depresses the response to A.C. but increases that to potassium and diminishes tone. 1 in 10^5 depresses all. 1 in 10^4 causes tonic contraction.

Action of bismuth.—1 in 10^7 increases the response to A.C. and potassium but decreases tone. 1 in 10^6 decreases the response to A.C. but increases that to potassium and tone. 1 in 10^5–10^4 depresses the response to A.C., and potassium but causes tonic contraction.

Action of chromium.—1 in 10^6 increases the response to A.C. and potassium. Higher concentrations (1 in 10^6–10^4) are depressant and decrease tone.

Action of uranium.—1 in 10^7 increases the response to A.C. and potassium but decreases tone. 1 in 10^6 increases the response to A.C. but decreases that to potassium and tone. 1 in 10^5 decreases the response to A.C. and increases that to potassium but diminishes tone. 1 in 10^4 is depressant.

Action of manganese.—1 in 10^7 increases the response to A.C. and potassium; 1 in 10^6 increases the former and decreases the latter. Higher concentrations (1 in 10^5–10^4) depressant.

Action of iron.—1 in 10^7 improves the response to potassium. 1 in 10^6 decreases the response to both and tone. 1 in 10^5 decreases the response to A.C. but increases that to potassium and decreases tone. 1 in 10^4 has the opposite effect on response to A.C. and potassium.

Action of cobalt.—1 in 10^7 decreases the response to A.C. and increases that to potassium and tone. 1 in 10^6 decreases the response to A.C. and potassium but increases tone. 1 in 10^5 has similar action. 1 in 10^4–10^3 is depressant and decreases tone.

Action of nickel.—1 in 10^7–10^5 decreases the response to A.C., but 1 in 10^4 increases it. Salant and Mitchell (1916) found that dilute solutions of nickel salts produce depression which may be followed by stimulation. 1 in 10^7–10^4 increases the response to potassium. 1 in 10^7–10^5 increases and 1 in 10^4 decreases tone.

Action on inhibition.—Zinc antagonises the inhibition produced by adrenaline (1 in 10^6) in the guinea pigs uterus in concentrations 1 in 10^7–10^4, but augments the potassium inhibition. It augments one kind of electrical inhibition and antagonises the other. If tonic contraction develops then it may antagonise all kinds of inhibitions. This is in agreement with the fact that protein precipitants may change an inhibitory into an excitatory effect (Sollmann, 1942).

Discussion

Many poisonous metals are beneficial in small concentrations; this reminds one of the Ayurvedic System of medicine. There is some relation between the electronic configuration of atoms and the physiological action. Thus in Groups I and II of the Periodic Table, the elements of subgroup *a* are comparatively less toxic than those of the subgroup *b*. Thus lithium, sodium and potassium are less toxic than copper, silver and gold. Similarly magnesium, calcium, strontium, and barium are less toxic than zinc, cadmium and mercury.

Toxic doses of metals cause tonic contraction. This is in agreement with the view mentioned previously that anything that injures the cell membrane would cause tonic contraction (Singh, 1944*b*). In small concentrations they cause inhibition. This is perhaps related to the action of some of them in destroying parasites *in vivo*. It is possible that they produce inhibition of some activity of the parasites and so make them susceptible to other agencies of the defence mechanism of the body. Thus it is known that *in vivo* these metals destroy parasites in much smaller concentrations than *in vitro*.

On unstriated muscle, the action of metals resembles those of other substances. Thus they may increase the excitability or decrease tone and *vice versa*, or they may differentially affect the excitability to A.C. and potassium and tone in an independent manner (Singh, 1944*a*).

PART II

Action of Water-Soluble Vitamins

Action of thiamine.—

In frog stomach.—1 in 10^7 increases the response to alternating current and spontaneous contractions, and does not significantly affect the response to potassium and tone. 1 in 10^6 then decreases the response to A.C. and increases that to potassium; tone decreases in muscles which are sensitive to electric current, but increases in those sensitive to potassium. Further concentrations (1 in 10^5–10^3) are depressant, but tone is affected as above.

In dog stomach.—1 in 10^7 greatly increases tone, so that the excitability is depressed both as regards A.C. and potassium. With 1 in 10^6, tone decreases, so that the response to A.C. slightly improves; this action increases with 1 in 10^5. 1 in 10^4 again increases tone. Small concentrations (1 in 10^7–10^6) thus cause contraction, and larger concentrations (1 in 10^5) relaxation.

Action of riboflavine.—

In frog stomach.—1 in 10^7 increases the response to A.C., and does not significantly affect that to potassium and tone; 1 in 10^6 then decreases the response to A.C. Higher concentrations (1 in 10^5–10^4) are depressant and decrease tone.

In dog stomach.—The action resembles that in the frog stomach. Small concentrations increase and large concentrations decrease tone.

Action of nicotinic acid.—

In frog stomach.—1 in 10^7 has no significant action. 1 in 10^6–10^5 increases the response to A.C. and potassium and does not significantly affect tone. 1 in 10^4 is depressant and decreases tone.

In dog stomach.—1 in 10^7 increases tone and decreases the response to potassium. 1 in 10^6 increases the response to A.C., potassium and tone. 1 in 10^5 then decreases the response to A.C., increases that to potassium and decreases tone. 1 in 10^4 was found to increase the response to A.C. but decrease the response to potassium and tone.

Small concentrations thus increase and large concentrations decrease tone, as a result of which the response to A.C. increases.

Action of ascorbic acid.—

In frog stomach.—Small concentrationa (1 in 10^7) are inhibitory to A.C., potassium and slightly to tone. 1 in 10^6 decreases the response to A.C. and potassium, though the response to latter may also increase. 1 in 10^5–10^4 are depressant though 1 in 10^4 may slightly increase the response to A.C.

In dog stomach.—1 in 10^8 improves the response to A.C., potassium and tone. 1 in 10^7 has the opposite action though the response to potassium may increase. 1 in 10^6 increases the response to A.C. and decreases that to potassium; tone is decreased. Further concentrations (1 in 10^5–10^4) are depressant to A.C. and potassium, though the action on tone is variable. Small concentrations (1 in 10^8–10^7) may increase and large concentrations (1 in 10^5) may decrease tone.

Action of hesperidin.—

In frog stomach.—1 in 10^7 decreases the response to A.C. and potassium and increases tone. 1 in 10^6–10^5 decrease the response to A.C. but increase that to potassium and tone. 1 in 10^4 increases the response to A.C. and potassium and decreases tone.

In dog stomach.—1 in 10^7 increases the response to A.C., and decreases that to potassium, and greatly increases tone. The response to potassium may be oppositely affected. 1 in 10^6 increases the response to A.C., and tone now decreases when compared to that with 1 in 10^7. These actions increase with further increase in concentration (1 in 10^5). 1 in 10^4 decreases the response to A.C. and tone, and may increase that to potassium.

Discussion

The action of water-soluble vitamins follows the rule already laid down for the action of other substances. The excitability to A.C. and potassium may be similarly or oppositely affected, and tonic contraction is depressant to both.

PART III

Action of Anæsthetics

These and subsequent experiments were performed on frog stomach.

Action of ethyl alcohol.—1 in 10^7 increases the response to A.C., decreases that to potassium and does not affect the tone significantly. I in 10^6 increases the response to A.C. but to a lesser extent, and further decreases the response to potassium. 1 in 10^5 is more depressant to A.C. than 1 in 10^6, less depressant to potassium and decreases tone. 1 in 10^4 increases the response to A.C. and potassium but decresaes tone. 1 in 10^3–10^2 decreases the response to A.C. but increases that to potassium and tone; 1 in 10 has similar but more powerful action.

Action of methyl alcohol.—1 in 10^7 decreases tone and the response to A.C. and increases that to potassium. 1 in 10^6 has similar effect. 1 in 10^5 increases the response to A.C. and potassium and decreases tone; further concentrations (1 in 10^4–10^2) decrease the response to A.C. and tone and increase that to potassium. 1 in 10 is depressant all round; it does not cause tonic contraction.

Action of amyl alcohol.—1 in 10^7 is depressant to A.C. and potassium but increases tone. 1 in 10^6–10^4 is depressant to A.C., but increases the response to potassium and decreases tone. Higher concentrations are depressant.

Action of glycerol.—1 in 10^7 is depressant. 1 in 10^6 increases the response to A.C. and potassium but tone is diminished. 1 in 10^5–10^2 decreases the response to A.C., and increases that to potassium; tone increases and 1 in 10 causes a marked tonic contraction.

Action of chloroform.—1 in 10^{7}–10^{6} increases the response to A.C., but diminishes that to potassium and tone. 1 in 10^{5}–10^{4} decreases tone and the response to A.C., but increases that to potassium. Higher concentrations are depressant.

Action of ether.—Exact concentration was difficult to note, but as found previously (Singh, 1938), a certain concentration decreases the response to A.C., and increases that to potassium.

Action of chloral hydrate.—1 in 10^{7} is inhibitory. 1 in 10^{6}–10^{4} decreases the response to A.C. and increases that to potassium, 1 in 10^{5}–10^{4} increasing tone as well. 1 in 10^{3} decreases tone, so that the response to A.C. may again increase. 1 in 10^{2}–10^{1} is depressant.

Action of procaine.—1 in 10^{7} is inhibitory: 1 in 10^{6} increases the response to A.C. and potassium and decreases tone. 1 in 10^{5}–10^{4} decreases the response to A.C., and increases that to potassium and tone.

Action of cocaine.—1 in 10^{7} increases the response to A.C. and decreases that to potassium and tone. 1 in 10^{6} decreases the response to A.C. and tone and increases that to potassium. 1 in 10^{5} is depressant. 1 in 10^{4} causes tonic contraction.

Action of morphine.—1 in 10^{7}-10^{6} increases the response to A.C. and decreases that to potassium; tone is not significantly affected. 1 in 10^{5}–10^{4} decreases the response to A.C. and tone and increases that to potassium; 1 in 10^{3} causes tonic contraction.

Discussion

Small concentrations of anæsthetics, as in the nervous system, may be inhibitory to unstriated muscle. The effect on the tone is variable depending upon the state of the muscle. Damaging concentrations increase the excitability to potassium and cause tonic contraction, and decrease the excitability otherwise.

PART IV

Action of Calcium Precipitants

Action of sodium sulphate.—1 in 10^{7}–10^{6} increases as excitability to A.C. and tone. 1 in 10^{5} increases the excitability to A.C. and potassium and tone. 1 in 10^{4}–10^{2} increases the excitability to A.C., decreases that to potassium and increases tone. This increase in tone is not due to inactivation of calcium, as the latter has opposite effect, and the calcium affected would be much too small. 1 in 10 sodium sulphate increases the excitability to A.C. and decreases that to potassium and tone. High concentrations are depressant.

Action of sodium citrate.—1 in 10^7 increases the response to A.C. and potassium and decreases tone. 1 in 10^6–10^4 increases the response to A.C. and decreases that to potassium and tone. 1 in 10^3 has similar action, but may increase the response to potassium. 1 in 10^2 increases the response to A.C. and tone and decreases the response to potassium. Higher concentrations are depressant but cause tonic contraction.

Action of sodium fluoride.—1 in 10^7–10^5 increases the response to A.C., but decreases that to potassium and tone. 1 in 10^4 increases the response A.C. and potassium and tone. 1 in 10^3 decreases the response to A.C. and tone and decreases that to potassium. 1 in 10^2 is depressant, but 1 in 10 causes tonic contraction. With replacement of about 5% of the chloride of the saline with other halides, the excitability to A.C. increases in the following order:—$F < Cl < Br < I$.

Action of sodium oxalate.—1 in 10^7–10^5 increases the response to A.C. and decreases the response to potassium and tone. 1 in 10^4 increases the response to A.C. and potassium and tone. Higher concentrations are depressant.

Discussion

Substances which remove calcium from activity affect the excitability of unstriated muscle by diminishing adaptation and by producing other effects of calcium deficiency, which may be antagonistic. The former effect will increase the excitability and the latter effect will be variable. Besides the ions have no doubt their specifications.

PART IV

Action of Some Other Substances

Action of sodium acetate.—1 in 10^7–10^2 has no significant action. 1 in 10 increases the response to A.C. and tone and decreases that to potassium replacement of the chloride of the saline with acetate increases the response to A.C. and tone and decreases that to potassium.

Action of sodium cyanide.—1 in 10^7–10^6 is inhibitory. 1 in 10^5 increases the respone to A.C. and decreases that to potassium and tone. 1 in 10^4–10^3 has opposite action. Further increase in concentration of the cyanide causes a marked tonic contraction.

Action of atropine.—1 in 10^7 increases the response to A.C. and decreases that to potassium and tone. Further increase in concentration is depressant.

Action of quinine.—1 in 10^7 increases the response to A.C. and decreases that to potassium and tone. Further increase in concentration (1 in 10^6–10^5) is depressant to A.C. and potassium but increases tone.

Action of formaldehyde.—1 in 10^7–10^6 increases the response to A.C. and decreases that to potassium; tone is not significantly affected. 1 in 10^5 has the opposite action. 1 in 10^4–10^2 are depressant.

Discussion

There are following variables in the excitability complex of unstriated muscle, and these are affected independently by various agencies. (1) Excitability to electric current. (2) Excitability to potassium. (3) Tone. (4) Adaptation and inhibition. (5) Excitability to drugs. (6) Excitability to nervous stimulation. Viscosity may also be an independent factor.

It is possible that drugs act on the cell membrane, and thus altering its permeability, the action being specific like the opening of a lock by its key. If the permeability is decreased then the cell may be deprived of some vital substance, thus decreasing its activity. This may result in atrophy or even death as in the case of parasites. Increase in permeability by allowing increased access to raw materials required by the cell metabolism may have an opposite action. This might explain the action of minute quantities of drugs and hormones. It is possible that toxic doses might act in another way. If the permeability is too much increased, then toxic substances might diffuse in and so damage the cell or essential substances may diffuse out of the cell, and thus impair its activity. Thus if the permeability is increased potassium inside the cell may be replaced by sodium.

Bromides and iodides are known to cause eruptions in the skin of the human being. Thus anions cause unstriated muscle to swell. If the capillary walls of this particular part of the skin have special affinity for the bromides and iodides, they will swell and become more permeable and so the eruptions will ensue. If the permeability is increased, then sodium chloride would produce similar effects; other substances may produce such effects. As calcium prevents the swelling it is possible that the increase in permeability may result from release of calcium, and its subsequent diffusion away from the cells, or changes in permeability may be produced by changes in ionised calcium in the cells. The specificity of a substance may lie in its capacity to alter the calcium conceptration of the cell membranes. This suggestion is supported by the fact that the substances may exhibit specificity in causing inhibition of unstriated muscle; the latter is identical with adaptation or accommodation which is presumed to be due to changes in the free calcium in the cells. These will lead to changes in permeability, which in turn will affect the activity of the cell as presumed. This hypothesis gives a common explanation for the diverse physiological phenomenon such as the action of drugs on the unstriated muscle, the action of hormones, etc.

Substances that cause damage to the muscle, cause it to contract. These may be classed as irritants. What is the physiological nature of irritation? This appears to be an increase in permeability as irritants are known to cause hæmorrhages from capillaries.

In unstriated muscle, I have not been able to find any difference between fatigue and adaptation, and as the latter is identical with inhibition, it appears that in isolated muscle fatigue, inhibition, adaptation or accommodation are identical. This shows that even in isolated muscle, overactivity is prevented by concomitant inhibition as in the case of voluntary effort; in the former case inhibition is myogenic and in the latter case neurogenic.

Substances may cause contraction in small and relaxation in large concentrations (Singh, 1945). When excitation takes place in muscle it contracts, but the excitation subsides owing to adaptation or increase in inhibitory state. The muscle then relaxes, but then adaptation takes place to inhibitory state (adaptation to adaptation, or accommodation to accommodation) so that excitation recurs. This results in spontaneous or rhythmic contractions. This view is supported by the fact that in the presence of calcium most substances may not produce a contraction. If the calcium concentration is decreased, then spontaneous contrations occur, and if the calcium concentration is still further decreased, then tonic contractions occur; similar phenomena was found to occur in frog heart. Therefore fatigue, inhibition, adaptation, or accommodation and refractory period are identical, *i.e.*, all inhibitory states are the same. Any substance that causes contraction will cause relaxation under suitable conditions. Of these conditions, two can usually be successfully employed; one is to use a weak stimulus, and the other is to cause the muscle to contract, or diminish the excitability by means of a drug. The above are the methods which have been used to show the presence of vasodilatory fibres; it is clear that these methods are of doubtful utility.

Most substances affect the tone according to the state of muscle. If the muscle is sensitive to A.C., they may cause relaxation; if it is sensitive to potassium they may cause contraction. Former state can be produced by stimulation with A.C. and the latter with potassium. It appears that stimulation with A.C. results in the increase of the inhibitory state, so that when the muscle is stimulated by another agency, the inhibitory state predominates over the excitatory. This is probably due to the fact that all stimulants give rise to both excitatory, and inhibitory states, and if the threshold inhibitory state is already increased, then on addition of the stimulant, the combined inhibitory action of the stimulant and the resting inhi-

bitory state of the muscle exceed in action the excitatory action of the stimulant, *i.e.*, the same reason which accounts for the contraction of the relaxed muscle and relaxation, of a contracted muscle by a stimulant (Singh, 1945). The above is in agreement with the fact that *Mytilus* muscles which adapt rapidly, react by relaxation to most substances that usually cause contraction.

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TONUS IN UNSTRIATED MUSCLE

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Tonus in unstriated muscle has been variously explained. It has been ascribed to a catch mechanism (Grutzner, 1904; Von Uexkull, 1912) to increase in viscosity (Bayliss, 1928; Winton, 1930; Bozler, 1931; Singh, 1938 *a* and other papers).

No single explanation of tonus can suffice, as there are distinctly two kinds of tone. Many observers have shown that tone diminishes in the absence of oxygen, while others have shown that there is no increase in the oxygen usage during contraction. Parnas (1910) made experiments which show that in the bivalve mollusc no increase in metabolism is to be detected during the contraction. Bethe (1911) investigated the question in another way and confirmed the views of Parnas.

The metabolism of the mammalina plain muscle in the relaxed and the tonic states has been directly compared by Lovatt Evans (1923): he found that there was no increase in the oxygen usage of the tissue when it was brought into the tonically contracted state by the use of drugs such as histamine, but on the contrary rather less oxygen was used; this was accounted for by the fact that the tonic muscle was shorter and therefore exposed less surface than relaxed muscle; a similar diminution in oxygen usage was seen when the muscle was shortened by reduction of its load. Singh and Rao (1940) however found in the thin flat muscle of the frog stomach that extension decreased the oxygen consumption; they found that the oxygen consumption decreased during the isometric contraction and increased during inhibition. Singh (1938 *a*) found that life is not necessary for the slow tonic relaxation of the plain muscle.

In the present research an attempt has been made to elucidate the mechanism of tonus in the unstriated muscle.

Tonus with Oxygen Usage

This is an ordinary contraction dependent upon the oxygen supply. It is due to ions in the saline as it is antagonised by increase of osmotic pressure. It has the following characteristics: it has superadded sponta-

neous contractions or the muscle relaxes spontaneously periodically (Singh, 1942); inhibition is facilitated.

The relation between tone and inhibition is shown by the following experiments. The stimulating power of the ions varies in the order

$$Cl < Br < NO_3 < I < SCN < CN.$$

Using the guineapig uterus, replacement of the chlorides of the saline with bromide increases tone as well as inhibition. The same may happen with nitrate, but with iodide and thiocyanate tone decreases. The explanation is given in the previous paper (Singh, 1945 *a*).

This kind of tone develops in steps from relaxed condition of the muscle. On the top of the tonic contraction, spontaneous contractions are superadded. It is antagonistic to electrical excitation, the sensitivity to which can be increased by increasing the osmotic pressure of the saline. The excitatory action of the substances is decreased while their inhibitory action is increased; the reason for this is given in another paper (Narayana and Singh, 1944). A substance may affect the inhibition produced by another substance by producing an antagonistic or potentiating inhibition or by changing tone. Thus in the guineapig uterus (Singh, 1945 *b*) small concentrations of potassium decrease the adrenaline inhibition by a direct antagonistic action; larger concentrations increase it by increasing tone, and still larger concentrations decrease it by greatly increasing tone.

The above tone is excited by stretch and often a constant length appears to act as stimulus, preventing the muscle from elongating. In this way I have seen a muscle kept at practically constant length for four hours automatically.

Tonus without Oxygen Usage

To explain the holding power of the unstriated muscle a "catch" or "ratchet" mechanism has been suggested (Grutzner, 1904; Von Uexkull, 1912). There is some evidence for such a mechanism. Often the movements of the muscle in one direction appears facilitated but retarded in the opposite direction (see Fig. 9 of Singh, 1938 *b*; Fig. 6 of Singh, 1943 *b*). The assymetric stretch and release curves also suggest a similar mechanism (Singh, 1938 *c*).

The action of distilled water on unstriated muscle (Singh, 1944) supports the above view. When the frog stomach muscle is placed in distilled water, it elongates actively. This elongation may amount to 200% of the original length and cannot be accounted for by mere expansion due to gain of water by the muscle. It occurs in living as well as dead muscle, so that it appears to be a property of the myosin molecules. It also supports the view that

muscular contraction is due to folding of myosin molecules as elongation will then be due to opposite action.

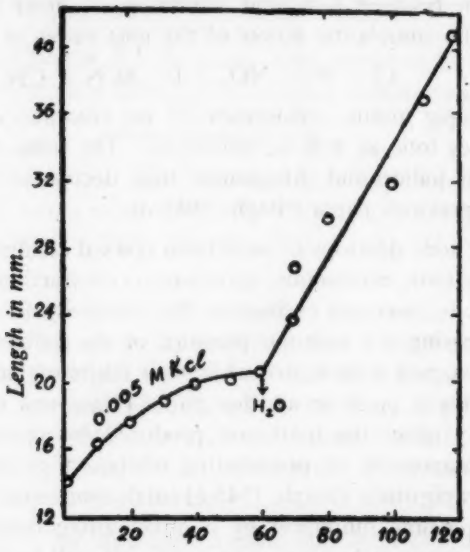

FIG. 1. Frog stomach. Effect of KCl on elongation

The addition of the ions to the water checks the elongation (Fig. 1). There is no significant difference between the actions of sodium and potassium chlorides; calcium chloride has a more potent action (Table I).

TABLE I

Effect of 0·01 *M salts on the elongation of the frog stomach*

Time in minutes	NaCl mm.	KCl mm.	$CaCl_2$ mm.
0	18·0	16·0	18·0
10	20·0	18·2	20·1
20	23·0	20·0	22·0
30	24·5	22·0	21·0
40	26·0	23·0	22·5
50	27·5	26·5	23·0
60	29·0	28·0	24·5
70	32·0	28·0	24·5
16 hours	39·0	34·0	27·0

The nearest approach to the physiological saline in which the muscle elongates significantly consists of 9 c.c. of 0·112 M KCl, 4 c.c. of 0·073 M $CaCl_2$, 5 c.c. of M/15 sodium phosphate at pH 7, 7 c.c. of 0·112 M NaCl, and water upto 100 c.c. (Table II).

TABLE II

Effect of mixtures of salts on elongation of frog stomach

Time in minutes	Number of muscles		
	1	2	3
0	17·0	19·0	17·0
10	19·0	21·5	20·0
20	20·0	22·5	21·5
30	20·5	23·0	21·5
40	20·5	23·0	22·0
50	21·0	23·0	22·0
60	20·5	23·0	22·5
16 hours	24·0	27·0	27·5

In doing the above experiments three narrow strips were cut transversely from the middle of the stomach, as the pieces from the pyloric and cardiac ends behaved irregularly. The narrower the piece the greater the elongation as the opposing force becomes less. One could see the separation of the longitudinal fibres by the elongating forces.

The cause of elongation must be opposite that of contraction. Distilled water would dilute the muscle contents, so that some constituents normally present in the muscle in combination with myosin is diminished in concentration and the forces of electrostatic attraction which it gives rise to are lessened, or the water molecules have the opposite effect. The substance responsible for the shortening of myosin is probably some ion, as shown by the action of ions on dead muscle (Singh, 1943 *c*). It is probably sodium (Singh, 1938 *a*, 1945 *a*); it must be a stable substance, otherwise energy will be required continuously during contraction.

An electrostatic system would give rise to a catch mechanism, but as mentioned, movement in direction of attraction will be facilitated but retarded in the opposite direction. This view fits in with the modern conception of the intimate nature of the muscular contraction as being due to folding of myosin molecules and suggests a common mechanism for tonic as well as twitch contractions; they have some common properties (Singh, 1946).

If substances combining with myosin (M) to produce tonic contraction be designated as T, and that to produce twitch or quick contraction be Q, then the compound MT would result in tonic contraction and MQ in twitch contraction. The chief difference between MT and MQ will be that the former will be a stable and the latter an unstable compound (Singh, 1943 *c*). The former will therefore not require a continuous increase in metabolism.

The instability of Q may be due to the fact that it may be continuously destroyed, an acetylcholine-like substance may be produced which is destroyed by an enzyme, or that Q may be simultaneously resynthesised into its former origin. Q may be adenosinediphosphate which is resynthesised into adenosinetriphosphate. T must be a stable compound.

Q and T appear to be mutually exclusive as shown by the fact that twitch and tone are antagonistic. Thus unstriated muscle may relax before it contracts, and so also striated muscle (Sandow, 1945).

There is probably a T acceptor C and a Q acceptor D. The formula for the twitch contraction would be

$$MT + C = M + CT$$
$$M + QD = MQ + D$$
$$MT + C + QD = MQ + D + CT$$

or it may be

$$MT + Q = MQ + T$$

D may be phosphate. T may be sodium as unstriated muscle is richer in sodium than striated muscle (Singh, 1938 *a*, 1945), and C is probably protein.

The above explains many of the tonic and twitch phenomena in unstriated muscle. The increase in viscosity found in the tonic contractions is probably a result and not the cause of them (Singh, 1938 *a*). MQ is accompanied by increase in viscosity (Gasser and Hill, 1942), so also MT (Singh, 1943 *a*). But if the viscosity of MQ is less than that of MT, then during twitch, the viscosity will descrease, as found by Winton (Singh, 1943 *a*).

The normal contractions of the unstriated muscle are probably mixtures of two to a varying degree (xMT + yMQ) where x and y are variables. If MQ predominates during contraction, then the oxygen consumption will increase, but if MT predominates, then it will decrease.

Summary and Conclusion

No single explanation of tonus in unstriated muscle can suffice as there are two kinds of tonic contractions, one with and the other without oxygen usage. The former is akin to ordinary contraction and the latter to some structural change in muscle. It is suggested that in tonic contractions without oxygen usage, myosin forms a stable compound with some ions in the muscle fibres, while in twitch such a compound is unstable. The two factors which combine with myosin to form the contractible compounds of tonic and twitch contractions respectively are mutually exclusive in their combination with myosin; this explains many tonic and twitch phenomena in unstriated muscle.

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